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INTRACRANIAL ANEURYSMS*

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The occurrence of multiple, true aneurysms of the intracranial arteries, of probable syphilitic origin, is sufficiently rare to warrant the report of such a case with a brief review of the literature.

The following case came to autopsy at St. Luke's Hospital, New York, on Sept. 23, 1927. The patient was in the medical service of Dr. Samuel W. Lambert.

REPORT OF CASE

History.—A woman, aged 57, of Irish nationality, was referred to the hospital from the outpatient department on Sept. 9, 1927, on account of retention of urine and cystitis. Because the patient was deaf and unable to read or write, it was practically impossible to obtain any sort of a history. She could relate nothing of her past illnesses. She stated that she had had difficulty in urination for about three weeks and had been catheterized several times before she was sent to the outpatient department. A history of venereal disease was not obtained.

Positive Physical Observations.—At the time of admission the patient was not in acute distress. The left pupil was slightly larger than the right; both pupils were slightly irregular and fixed to light. The ocular movements were normal. Deafness to the spoken voice was the only note concerning the ears. The heart was slightly enlarged, but the sounds were of good quality and murmurs were not present. The pulse rates were regular and equal, and the vessel walls could not be felt. Examination of the abdomen showed that the bladder was enlarged and tender and extended half way to the umbilicus. There was a tremor when the patient touched the nose with the finger, but no greater inaccuracy was observed. The Romberg sign was negative. She could walk a straight line but she did it inaccurately. The automatic associated movements were present and normal. Both patellar reflexes were hyperactive. The other reflexes were normal. The blood pressure was 120 systolic and 75 diastolic.

Laboratory Observations.—Examination of the urine revealed a trace of albumin, a quantity of pus cells and a few red blood cells. The blood showed a slightly increased percentage of hemoglobin and red cells and a white cell count of 11,000, with a normal differential count. The blood urea nitrogen on admission was 59 mg. per hundred cubic centimeters and the uric acid nitrogen 5.7 mg. per hundred cubic centimeters. The Wasserman reaction of the blood was negative with the acetone antigen, and plus-minus with the cholesterinized antigen. Lumbar puncture done on the day following admission showed a clear fluid under a pressure of 40 mm. of water. It showed thirty cells per cubic millimeter, all lymphocytes. The butyric acid reaction for albumin was two plus, and the Wassermann reaction was four plus. The colloidal gold curve was 11122.5331.510.5.

Treatment and Course.—The patient ran a temperature from 98.6 to 101 F. She could not void urine and was catheterized twice daily. About 30 ounces

* From the Pathological Laboratory, St. Luke's Hospital.

(887.25 cc.) of urine was obtained each time. Medications consisted first of oil of santol and then of methylene blue for the cystitis and of increasing doses of potassium iodide for the syphilis. Small doses of digitalis were also given. She became drowsy, restless and weak, and on September 23, fourteen days after admission, she suddenly collapsed and died.

Protocol of Autopsy.—(Abstract of Positive Observations Only).—The body was obese, measuring 157 cm. in length. It was cold, and rigor mortis was present. The pupils were in mid-dilatation and were about equal, but both were irregular in outline. On the lower part of the right leg old healed varicose ulcers were seen.

The lungs did not show anything abnormal except moderate congestion at the bases. The pulmonary arteries of both lungs showed irregular, yellowish gray thickenings of the intima.

The heart was fatty and the muscle of the left ventricle was pale, soft and flabby. All of the valves showed thin, free leaflets. The arch of the aorta showed raised, almost confluent, pearly white plaques, with intervening fine longititudinal striations. Macroscopic calcification was not present. These lesions extended downward to the border of the aortic valve. The coronaries showed a few yellowish plaques, but they were relatively normal. The aortic valve measured 8 cm. in circumference. The thoracic portion of the aorta showed the same type of lesions as those in the arch, except for a few superimposed yellowish calcified plaques. The thoracic portion measured 6 cm. in circumference. At the level of the twelfth thoracic vertebra the lumen became smaller and measured only 3.5 cm. in circumference.

The peritoneum did not show any lesions. The liver was bound to the under surface of the diaphragm by three bands of fibrous tissue. It cut with increased resistance. Section showed a fatty, congested organ with accentuated markings.

The spleen was adherent to the diaphragm. It was soft, tore easily and showed accentuated lymphoid markings and subcapsular hemorrhages.

The right kidney weighed 125 Gm. The capsule was thin, and it was stripped from the kidney with difficulty, bits of the cortex being torn away. The cortex was of normal thickness, and the markings were distinct. The pelvis was deeply stained with methylene blue. The left kidney was the same except that a large amount of cellular débris was present in the pelvis. Stained preparations showed only desquamated epithelium.

The bladder was collapsed. The wall was thick and edematous. The mucosa was in deep folds, was thickened and hemorrhagic and showed numerous white plaques of fibrin.

Lesions were not observed on the calvarium. There was a moderate thickening of the dura and pia-arachnoid over the cerebrum. At the base of the brain the pia-arachnoid was thickened and opaque. The right internal carotid artery, as it emerged from the cavernous sinus, showed a fusiform aneurysmal dilatation 1.5 by 1.2 by 1.2 cm. The adjacent portions of the anterior cerebral arteries were slightly dilated. At the same place there was a similar dilatation of the left internal carotid artery measuring 2 by 1.7 by 1.2 cm. The vertebral arteries showed larger lumina than they normally should. Just after their union there was an aneurysmal dilatation of the basilar artery, 2.5 by 2 by 1.5 cm. A second aneurysm of the basilar artery was seen at the point at which the posterior cerebral arteries were given off. It measured 2 by 1.8 by 1.2 cm. These two aneurysms of the basilar artery were partially filled with laminated blood clot. All the smaller vessels showed numerous yellowish plaques in their walls which at some points appeared to be associated with slight dilatation of the lumen (figs. 1 and 2).

The pituitary gland and the substance of the brain and brain stem did not show macroscopic lesions.

Microscopic Observations.—Sections of the heart showed a widespread atrophy of the muscle fibers. The fibers were separated by areas of acellular tissue and contained large vacuoles.

The lungs showed slight congestion throughout. Many alveoli contained desquamated respiratory epithelium.

Fig. 1.—Ventral aspect of the brain, showing opaque meninges and the aneurysms of the basilar and internal carotid arteries.

The liver cells showed diffuse fatty and granular degeneration. The sinusoids were congested and secondary atrophy of the cells was seen. An infiltration with round cells existed throughout.

The spleen was congested and showed an increase of the connective tissue and an aplasia of the lymphoid follicles.

The kidneys showed small wedges of connective tissue growing downward into the kidney substance replacing tubules and glomeruli. A few of the latter were hyalinized. The vessels were relatively normal. Small collections of round cells were observed beneath the capsule.

The pancreas showed a diffuse increase in the interstitial connective tissue. There was a diffuse round cell infiltration throughout the suprarenals.

The lining epithelium of the bladder was largely desquamated. The mucosa was greatly infiltrated with lymphocytes and polymorphonuclear leukocytes.

The intima of the aorta was thickened and showed numerous small collections of round cells in its substance. There was a myxomatous degeneration of the media. The vasa vasorum were surrounded by round cells. In the outer portion of the media, a proliferation of the capillaries was seen.

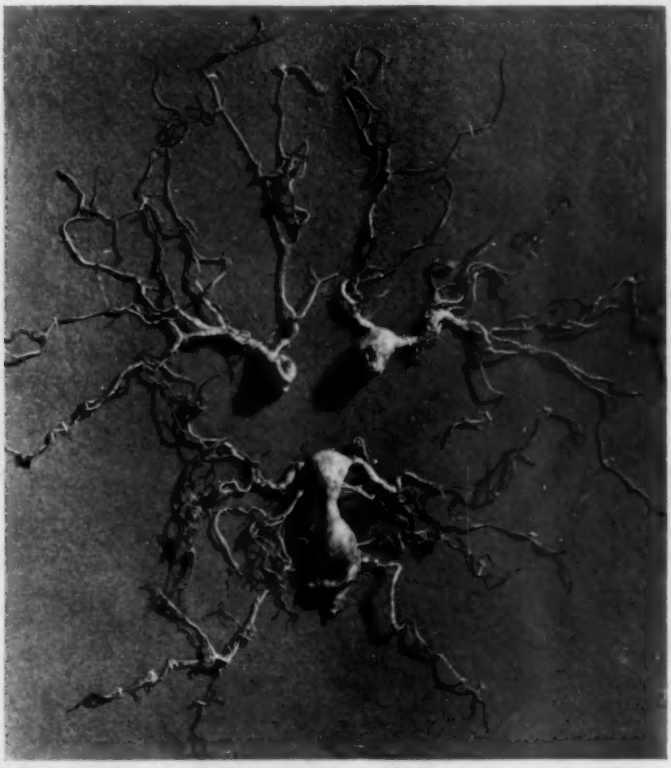

Fig. 2.—Dissected intracranial arteries, showing the aneurysms and the smaller lesions in the branches.

Sections from the aneurysm of the basilar artery showed a thin hyaline wall, containing in places small isolated collections of round cells (fig. 3).

There was a round cell infiltration of the pia-arachnoid over the vertex which extended deeply into the sulci along the vessels. Portions of the cerebrum showed softening around the vessels, the Virchow-Robin spaces being broken down (fig. 4). Lymphocytes and probably glial nuclei were found in these spaces. The cerebellum did not show any lesions.

Degenerative lesions were seen most clearly in the region of the posterior roots of the spinal cord, with a loss of axis cylinders and containing degenerated

ganglion cells from the adjacent nuclei. An increased number of capillaries were found in this region. The glial reticulum was irregularly granular. There was a round cell infiltration of the pia over the surface and in the sulci (fig. 5).

By starting with a definition of the subject under discussion, certain misunderstandings may be avoided at the outset. By the word aneurysm is meant a true, macroscopic dilatation of an artery, the wall of which is made up of one or more of the three anatomic coats, although these may be altered in a greater or less degree. Thus, there

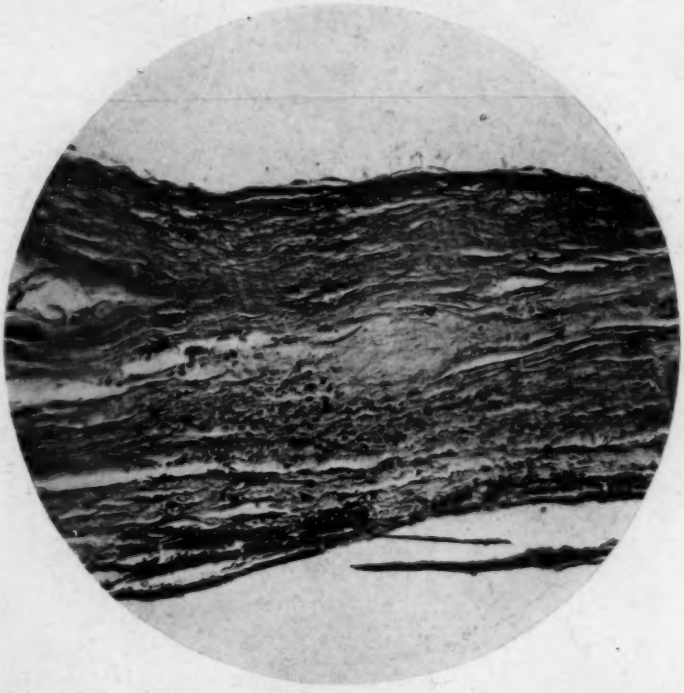

Fig. 3.—Photomicrograph of a portion of the wall of the aneurysm of the basilar artery.

are excluded from discussion all sacs caused by an arteriovenous communication, and all microscopic dilatations said so often to be precursors of cerebral hemorrhages. The word intracranial, instead of cerebral, has been used throughout as a qualifying word, to avoid confusion between all arterial aneurysms of the brain and those of the anterior, middle and posterior cerebral arteries themselves. In the past the word cerebral has been used loosely, in this connection, to describe aneurysms of the internal carotid, vertebral, basilar and cerebellar arteries, and the exact meaning in certain cases is not always clear.

Peacock [1] pointed out that probably the first reference in the literature to intracranial aneurysms was when "Morgagni, in alluding to the death by apoplexy in 1714 of Bernadin Ramazzini, suggests that the symptoms might have been caused by the rupture of an aneurysm of the arteries of the brain, founding this opinion on the circumstances that Ramazzini had for some years had a small aneurysm in each hand between the finger and the thumb." The first authentic account was published by Biumi [2] of Milan in 1765. He described an aneurysm

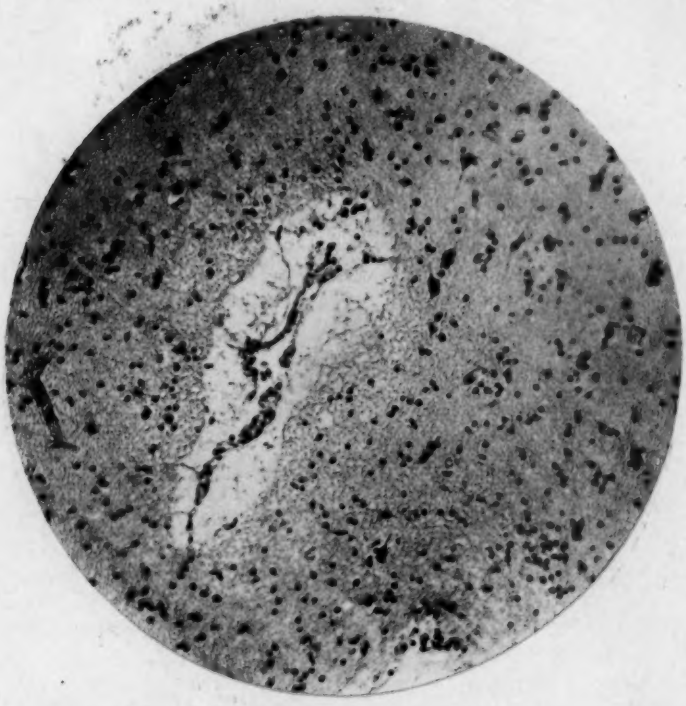

Fig. 4.—Photomicrograph of a portion of the cerebral cortex, showing softening around a small vessel and widening of the Virchow-Robin space.

of the internal carotid artery in a woman who had been suffering from attacks of rheumatic fever. Since then numerous single cases have been reported, and collections of these reported cases have appeared from time to time. The earlier contributions dealt with the more anatomic features, while the chief interest during the latter years has been with the symptomatology and diagnosis. This is in keeping with the whole trend of modern medicine.

1. Peacock, T. B.: St. Thomas' Hosp. Rep. **7**:119 and 317, 1876.
2. Biumi, quoted by Fearnsides, E. G.: Brain **39**:224, 1916.

Clinically, aneurysms of the intracranial vessels are considered rare and are scarcely mentioned in the differential diagnosis of intracranial lesions. That they are not actually rare is attested by the reports of large series of autopsies. Thus, Pitt [3] found twenty-three such aneurysms in 9,000 autopsies, Conway [4] forty-three in 6,325, Fearnsides [5] fifty-five in 5,432, and Osler [6] twelve in 800 necropsies. More recently Sossman [7] reported eight in 581 brains examined at the Peter Bent Brigham Hospital in Boston.

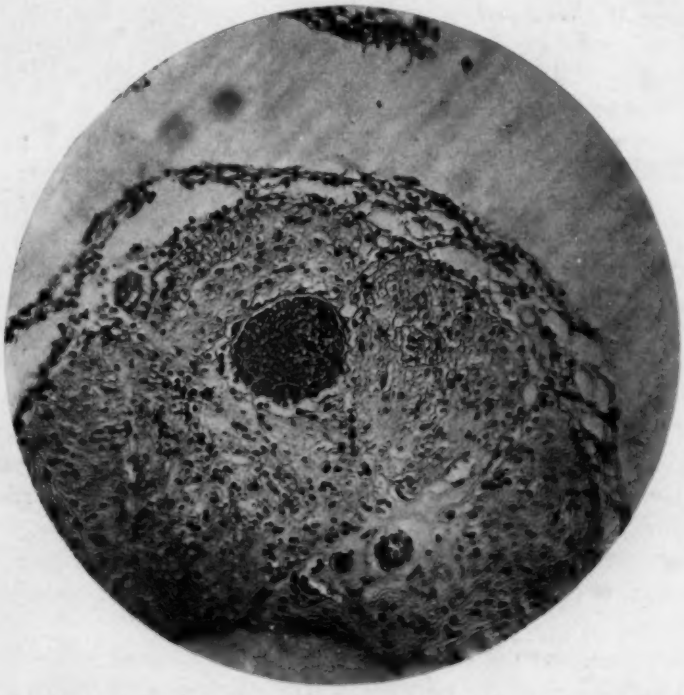

Fig. 5.—Photomicrograph of one of the posterior lumbar nerve roots, showing infiltration of the sheath and the nerve.

The anatomic sites of these dilatations have been tabulated by various authors. In 1859, Gull [8] analyzed sixty-two cases, and in 1876 Peacock [1] reviewed eighty-six. The similarity in their reports may

3. Pitt, Newton: Brit. M. J. **1**:827, 1890.

4. Conway, J. A.: Brit. J. Ophth. **10**:78, 1926.

5. Fearnsides, E. G.: Brain **39**:224, 1916.

6. Osler, Sir William: Principles and Practice of Medicine, ed. 8, New York, D. Appleton & Company, 1916, p. 1003.

7. Sossman, M. C.: Am. J. Radiol. **15**:122, 1926.

8. Gull, W.: Guy's Hosp. Rep. **5**:281, 1859.

possibly be due to the fact that with the exception of a few added cases, Peacock collected from the literature a great many of the same ones as did Gull. Peacock's observations in eighty-six cases were as follows:

Carotid Arteries and Branches	
Internal carotids	12
Arteriovenous	1
Anterior cerebral	4
Anterior communicating	5
Middle cerebral and branches	27
Total	49
Vertebral Arteries and Branches	
Vertebrals	5
Basilar	22
Cerebellar	3
Posterior cerebral	6
Multiple (small)	1
Total	37

The cause for this striking preponderance of occurrence of aneurysms in the internal carotid, middle cerebral and basilar arteries has not been clearly defined. The sudden release of the bony support of the carotid arteries as they enter the cranial cavity has been suggested as an etiologic factor in their special case. This is at least reasonable and cannot be refuted. It would seem that the volume flow in these larger arteries, plus their greater tendency to show degenerative lesions in their coats, might have a direct relationship to the formation of aneurysms.

The dimensions of these aneurysms have usually been small, many times being described as being the size of a pea or a cherry seed. The larger ones are most often seen on the basilar and internal carotid arteries, where they have been described as measuring from 30 to 40 mm. in diameter. It would seem from the reported cases that the actual size of an aneurysm may have little relation to the symptoms, large ones sometimes giving rise to no cerebral symptoms.

The only recorded case of multiple, moderately large intracranial aneurysms is the one reported by Bourneville.[9] The patient had dilatations of the right vertebral, anterior cerebellar and right and left posterior communicating arteries, which measured 1 by 0.6 cm., 0.7 by 0.6 cm., 0.6 by 0.4 cm. and 1 by 0.8 cm., respectively. The presence or absence of syphilis in this case is not mentioned.

9. Bourneville: Bull. Soc. anat. de Paris **43**:449, 1868.

ETIOLOGY

The etiologic factors concerned with aneurysmal dilatation of the intracranial arteries can be divided into the arteriosclerotic, syphilitic, embolic, traumatic and the less definite, called for want of a better name, congenital factors.

Arteriosclerosis: A detailed discussion of the pathology of arteriosclerosis need not be entered into here. Patients without clinical or serologic evidence of syphilis, in whom the degenerative changes are the essential lesions found in the intima and media of the muscular and elastic arteries, must, of necessity, be classed as arteriosclerotic. Infiltration with round cells as seen in inflammatory lesions is lacking. Of all intracranial aneurysms, by far the greater number are dependent on simple arteriosclerotic changes in the vessel walls. Thus, Turnbull [10] in summarizing his discussion says that "saccular aneurysms, due to medial degeneration independent of inflammation, are found more commonly in the cerebral arteries than on any other of the muscular arteries."

Syphilis: The relationship of syphilis to intracranial aneurysms has always been much discussed, especially since the Wassermann test is so widely used. It is in this connection that the word cerebral has been most often misused and misunderstood. I have been unable to find in the reported cases in the literature a single case of true aneurysm of one of the cerebral arteries which was of definite syphilitic origin. On the other hand, syphilitic dilatations of the vertebral and basilar arteries have been reported in a great many cases. In recent years the reports of Boinet [11] and Krabbe and Backer [12] especially stressed this relationship. Fearnsides [5] in trying to explain this unusual distribution of syphilitic aneurysms suggested that "the relatively high incidence of syphilitic aneurysm of the basilar artery is probably due to the fact that this artery is a large one and that in the smaller cerebral arteries, syphilitic inflammation usually leads to such a severe degree of intimal hypertrophy that the lumen of the artery is blocked and thus prevents aneurysms." As a working theory this is tenable, even though definite proof is lacking. In Fearnsides' series of forty-four cases he found none in which the degenerative lesion in the arterial wall was the result of the inflammatory reaction usually associated with syphilis. Sossman [7] reported the case of one patient with a positive Wassermann reaction and suggested that 5 per cent of intracranial aneurysms are due to syphilis. It may be said in summary then that syphilis as a cause of intracranial aneurysms usually manifests itself in the vertebral and basilar arteries and as a factor in the sum total of intracranial aneurysms it plays only a small rôle.

10. Turnbull: Quart. J. Med. **8**:201, 1915.
11. Boinet, E.: Séances et mém. Soc. biol. **69**:210, 1910.
12. Krabbe, K. H., and Backer, K. H.: Acta med. Scandinav. **56**:95, 1922.

Embolism: Many intracranial aneurysms have been found in patients who at the time either were suffering from acute endocarditis or showed evidence of an old process on the heart valves. In the absence of other definite etiology, the inference has been naturally drawn that they are related as cause and effect. Church [13] in 1870 first stressed this relationship and collected thirteen cases, all occurring in patients under 20 years of age. With the exception of two on the basilar and one on the left internal carotid artery, they were all situated on the cerebral and communicating arteries. Wichern [14] in his twenty-two cases interpreted five as being due to emboli. In none of these, however, is any definite evidence of the relationship between the infectious lesion on the heart valves and the aneurysm given. That such mycotic aneurysms of the intracranial arteries do not occur cannot be denied, but the relation between the two is one of inference, strengthened by the absence of other definite etiologic factors, rather than that of pathologic or bacteriologic proof.

Trauma: Trauma as a cause of intracranial aneurysms is of rare occurrence and need not be more than mentioned. Kirby [15] reported an aneurysm of the right internal carotid artery just after its exit from the cavernous sinus, in a man who had suffered a fracture of the skull thirty-four years previously. He concluded that the aneurysm was the result of the fracture, even though evidence of the latter was seen only in the left sphenoid bone while the aneurysm was of the right internal carotid artery. Other reported cases have even less definite relationship to supposed etiologic trauma.

Congenital Factors: There remains a small number of cases which do not fall into any of the preceding groups. These are usually seen in very young patients, without syphilis, arteriosclerosis or endocarditis, and are considered to be due to a congenital weakness in the wall of the vessel. Eppinger [16] in his discussion of aneurysms and the pathology of arteries in general, was of the belief that many aneurysms of the smaller arteries are due to such a defect in the elastic properties of the walls. This is especially seen at their points of bifurcation. As this group is composed of those cases without a really definite pathologic histology, it has been used as a convenient one in which to cast all cases of unknown etiology.

AGE

The etiology of intracranial aneurysms naturally affects the age incidence of their occurrence. Since the majority are dependent on the arteriosclerotic changes associated with middle and old age, by far

13. Church, W. S.: St. Barth. Hosp. Rep. **6**:99, 1870.
14. Wichern: München. med. Wchnschr. **58**:2224, 1911.
15. Kirby, D. B.: Am. J. Ophth. **7**:577, 1924.
16. Eppinger: Arch. f. klin. Med., 1887, vol. 35.

the greater number of aneurysms are seen during this period of life. The following fifty-eight cases were analyzed by Gull [8] according to the age incidence:

Years	No. of Cases
Under 25	12
25 to 40	13
40 to 60	29
Over 60	4
Total	58

Conway [4] in his analysis of forty-three cases found approximately the same age incidence.

SEX

It has long been well known that aneurysms of the aorta, both in the arch and in the abdominal portion, are much more common in males than in females. This ratio of males to females has been given by Osler [6] as 5:1 for aneurysms of the aortic arch and 8:1 for those of the abdominal portion. Such is not true of intracranial aneurysms. In Hofmann's [17] seventy-three cases, fifty-one occurred in females and twenty-two in males. However, this is the only series in which the cases in females outnumbered those in males. In the reports of Fearnsides and Osler, the cases in males numbered slightly more than half. This more even distribution of intracranial aneurysms between the sexes is probably dependent on the main etiologic factor, arteriosclerosis, which affects both sexes in equal numbers.

DIAGNOSIS

Only the most salient of the clinical features will be mentioned here. Conway [4] has tersely given the following four reasons why the possibility of an intracranial aneurysm rarely suggests itself when cerebral symptoms are considered:

1. Aneurysms may exist and not give rise to any symptoms that can be recognized clinically.
2. They often cause sudden serious symptoms, followed by death, without affording an opportunity for the study of prodromal symptoms.
3. Age does not give any clue as to the presence or absence.
4. There is not any particular diathesis or constitutional state which favors the condition or would lead one to suspect aneurysm against neoplasm.

Beadles [18] in 1907 collected 441 cases from the literature and added 114 seen by him in museums in London and elsewhere, making a total of 555. This large series was especially analyzed according to the symptomatology shown. He divided the cases into four groups:

17. Von Hofmann, E.: Wien. klin. Wchnschr. **7**:823, 1894.
18. Beadles, C. F.: Brain **30**:285, 1907.

(1) those in which the first indication of a cerebral lesion was a fatal apoplectic attack due to rupture, numbering 257, or 46.3 per cent of the series; (2) those in which fatal apoplexy was preceded by symptoms suggesting a cerebral tumor or other cerebral lesion, numbering 115, or 20.7 per cent; (3) those in which there had been symptoms of cerebral tumor only, numbering 91, or 16.39 per cent, and (4) those without any cerebral symptoms, in which the aneurysms were accidentally discovered after death, numbering 92, or 16.61 per cent. It will be seen then that an apoplectic attack, followed either by recovery or by death, is a common feature in the symptomatology of intracranial aneurysms, occurring in 67 per cent of Beadles' series. As this symptom is dependent on hemorrhage, there is little to distinguish it from a ruptured vessel without aneurysm.

The symptoms dependent on nerve compression or irritation assist in the localization of a lesion, but at the same time help little in the differential diagnosis from tumors. Symptoms referable to the second, third, fourth, fifth and sixth nerves are of common occurrence. Deafness in patients with basilar aneurysms, such as those in the case recorded here, has repeatedly been reported, although the lesion is some distance from the eighth nerves.

A murmur, heard either by the patient or by a second person listening over the cranium, has often been used as positive diagnostic proof of the existence of an aneurysm. Beadles pointed out that patients with aneurysmal-like murmurs in life have at autopsy been found to have gliomas, extensive softenings and simple anemia, instead of aneurysms. He also said that murmurs are often heard in children in association with rickets, chronic hydrocephalus and anemia, and sometimes in perfectly normal persons. He could find only two cases in which a murmur was heard during life in a case of true, uncomplicated intracranial aneurysm. One of these aneurysms was of the vertebral artery and the other was of the cavernous portion of the internal carotid artery. In this connection there is an interesting report of a case in which the patient was operated on by Sir Victor Horsley.[19] On opening the skull the surgeon found a large pulsating tumor on the under surface of the brain. It sprang from the right internal carotid artery immediately after the dura was pierced and measured 1¾ inches (4.4 cm.) in diameter. It was cystic to the touch. Being interested in the murmurs said to be present in such aneurysms, the surgeon applied a sterilized stethoscope to the pulsating tumor and did not hear any murmur. He ligated the left common carotid artery in the neck. When last seen the patient had been well for five years. As will be mentioned later,

19. Horsley, Sir Victor, quoted by Beadles (footnote 18).

this is one of the rare cases in which definite therapeutic effects have been obtained in the treatment of a proved aneurysm inside the cranial cavity.

In reading numerous case reports, one is impressed by the similarity of the signs and symptoms due to small hemorrhages from aneurysm situated at the base of the brain. The apoplectic attack is often preceded by such prodromal symptoms as headache and dizziness, and at the time of the hemorrhage patients complain of something snapping at the base of the brain. A great many of the first attacks are fatal. Patients who have recovered from characteristic attacks complain of severe pain in the back of the neck and a sense of fulness in the back of the head. They are often irrational. The spinal fluid is said to be bloody. Symonds [20] reported five cases diagnosed during life, in two of which this same train of symptoms was observed. However, the diagnosis was not confirmed anatomically in either case. In one case an autopsy was not performed; in the other a hemorrhage was found extending into the brain substance, but the specimen was discarded before a careful search could be made for the aneurysm. Symonds concludes, however, that a history of a basal hemorrhage with signs of a tumor at the base of the brain is sufficient evidence for a diagnosis of aneurysm.

Careful study of routine roentgenograms of patients with cerebral symptoms has in a few cases led to a tentative diagnosis of aneurysm of one of the intracranial arteries. Many more plates can be so interpreted in retrospect, following the finding of aneurysms at operation or autopsy. Odqvist [21] reported a case of aneurysm of the basilar artery which was revealed by the roentgen ray and diagnosed as a tumor. He called especial attention to the presence of striae and fine calcified plaques deposited in the periphery of the shadows and seen as curved lines. Such a picture depends on calcification in the wall of a fairly large aneurysm. Erosion of bone, such as may occur with a pulsating aneurysm of the internal carotid artery, is suggestive, but is not diagnostic, of such a lesion. Sossman [7] says that in his series "in 25 per cent of the cases in which skull films were secured the diagnosis was possible from the roentgen examination and that in another 25 per cent, our findings would support the clinical findings."

Concerning the diagnosis of these lesions, no better conclusions can be drawn now, twenty years later, than those of Beadles [18] in 1907 after his study of 555 cases. He says, "The conclusion which I am forced to make from a careful study of a large series of cases is, therefore, that it is quite impossible to diagnose an aneurysm of

20. Symonds, C. P.: Guy's Hosp. Rep. **73**:139, 1923.

21. Odqvist, H.: Acta med. Scandinav. **63**:286, 1925.

any one of the cerebral arteries except in the most unusual circumstances. I will go further and say that in the vast majority of cases of aneurysm, a tumor, even, cannot be diagnosed. If there is any one sign to which special attention might be drawn, it is the occasional intermittent character of the symptoms."

TREATMENT

Of scientific and not real practical interest are the isolated reports of attempts to cure patients with intracranial aneurysms. The often quoted case in which Coe [22] cured a patient of certain cerebral symptoms by ligation of the common carotid artery, and that in which Humble [23] obtained a spontaneous cure of an intracranial aneurysm after the administration of iodides, cannot be seriously considered. The presence of aneurysm was not confirmed by autopsy or operation in either case. Sossman reported a case observed at the Peter Bent Brigham Hospital in which the cranial cavity was explored for a tumor. An aneurysm of the right internal carotid artery was encountered. A ligature was laid in place, the sac was opened and a laminated clot was removed in an attempt to do an aneurysmorrhaphy. Removal of the clot was followed by a profuse hemorrhage which necessitated tying of the ligature. The cavity was filled with muscle, and the sac was sewed up. A left hemoplegia resulted which gradually cleared up, with only a partial return of sensation. The patient was discharged in an improved condition, but he died at home six months later. An autopsy confirmed the surgical observations.

Magnus [24] reported a similar case in which the symptoms led to a clinical diagnosis of tumor in the right trigeminal region. At operation a sac the size of a chestnut was encountered. It contained blood coagulum. A huge hemorrhage, which was stopped only by ligation of the right internal carotid artery, occurred following the removal of the coagulum. The trigeminal ganglion on that side was removed. In three days the left hand became paralyzed except for a slight ability to move the fingers. The upper part of the arm was normal. The patient was discharged improved. A follow-up record was not given.

The two cases just described and the one reported by Sir Victor Horsley are the only ones found in the literature in which the presence of an intracranial aneurysm was verified by operation or autopsy, and in which therapeutic measures directed toward the cure of such an aneurysm were at all effective.

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22. Coe, R. W.: Ass. M. J. **3**:1067, 1855.

23. Humble, W.: Lancet **2**:874, 1875.

24. Magnus, V.: Aneurysm of Internal Carotid Artery, J. A. M. A. **87**: 1712 (May 28) 1927.

SUMMARY

In the light of the foregoing comment, the case reported here shows several interesting features. Clinically, the patient had meningovascular syphilis with changes in the spinal cord, but there was not any indication of either an intracranial aneurysm or a tumor. The deafness and low mental capacity can be explained in other ways more easily than by the presence of aneurysms. As purely accidental observations, two fusiform aneurysms of the basilar and one each of the right and left internal carotid arteries were seen at autopsy. Evidence of bone erosion or compression of the brain substance was not found. Microscopic sections of the wall of the basilar aneurysm showed, besides diffuse hyaline degeneration, small collections of round cells in the media. These were interpreted in this case, in the light of the other observations, as evidence of the inflammatory reaction associated with syphilitic arterial disease.

CONCLUSIONS

1. Macroscopic, true, arterial intracranial aneurysms are found in about 1 per cent of the brains examined at autopsy.

2. They are usually single and are seen most often on the basilar, internal carotid and middle cerebral arteries.

3. Syphilis as the cause of intracranial aneurysms usually manifests itself in the vertebral and basilar arteries, and as a factor in the sum total of intracranial aneurysms it plays only a small rôle.

4. The majority of intracranial aneurysms showing definite pathologic lesions are due to arteriosclerosis, while others with less definite lesions have been ascribed to infected emboli and a congenital weakness in the vessel walls.

5. Repeated hemorrhages from aneurysms at the base of the brain give rise to the most characteristic clinical symptoms, but a diagnosis of intracranial aneurysm is rarely warranted.

SPLENIC MYCOSIS *

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In recent years attention has been called by numerous investigators to the occurrence in certain spleens of peculiar yellowish-brown, firm nodules located in the trabecles. They are spherical or irregular in shape and vary in size, some being just visible to the naked eye, and others having a diameter of several millimeters. By fusion, larger, infarct-like areas may result. The color of these nodules is formed by deposits of different kinds of pigments, most of which contain iron. One pigment is of special interest. It is a light yellowish-green and forms branched trabecles which resemble shrubs or bushes. The pigment is embedded with sclerosed connective tissue and thickened elastic fibers, and is also taken up by foreign body giant cells. In some nodules the pigmentation is combined with calcification. The whole area is surrounded by recent hemorrhages.

These iron nodules were first described by Gandy in 1905, who observed them in the spleen of a patient with biliary cirrhosis. Later studies, especially those by Christeller and Puskepellis,[1] Eppinger,[2] Hennings,[3] Klinge,[4] Kraus,[5] Lubarsch,[6] Rotter,[7] Schuppisser [8] and Siegmund,[9] dealt with the microscopic structure of the nodules and with the microchemical reactions of the pigments. The yellowish-green trabecles have also been seen outside the spleen, by Lubarsch in an ovary and by Schuppisser in an adenomatous goiter.

The spleens which contain the intratrabecular areas of siderosis are often markedly enlarged, but in some instances they are smaller than the normal organ. They have been observed in cases of hemolytic

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1. Christeller, E., and Puskepellis, M.: Virchows Arch. f. path. Anat. **250**: 107, 1924.

2. Eppinger, H.: Die hepato-lienalen Erkrankungen, Berlin, Julius Springer, 1920.

3. Hennings, K.: Virchows Arch. f. path. Anat. **259**:244, 1926.

4. Klinge, F.: Virchows Arch. f. path. Anat. **255**:599, 1925.

5. Kraus, E. J.: Beitr. z. path. Anat. **70**:234, 1922.

6. Lubarsch, O., in Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1927, vol. 1, part 2, p. 480.

7. Rotter, W.: Virchows Arch. f. path. Anat. **259**:631, 1926.

8. Schuppisser, H.: Virchows Arch. f. path. Anat. **239**:320, 1922.

9. Siegmund: Centralbl. f. allg. Pathol. u. path. Anat. **33**:207, 1922.

jaundice (Eppinger, Christeller and Puskepellis, Oberndorfer and Siegmund), Banti's disease (Siegmund), syphilis (Hennings and Christeller and Puskepellis), atrophic cirrhosis of the liver (Eppinger), portal thrombosis and other disturbances of the portal circulation (Christeller and Kraus), leukemia (Kraus and Aschoff) and Hodgkin's disease (Schuppisser).

Gamna [10] thought that the spleens with the nodules, the color of which he compared with tobacco leaves, represent a new type of splenomegaly belonging to the specific granulomas. The etiology of this granuloma is unknown. He suggested the name "siderotic splenogranulomatosis," and was particularly interested in the giant cells.

French investigators recently advanced a new explanation of the iron nodules. Nanta, Pinoy and Gruny,[11] studying a form of anemia with splenomegaly which is common among the younger men of Algiers, believed that this disease is caused by a fungus. The large spleens contain the siderotic areas in which Nanta and his co-workers found branched and segmented filaments besides various undetermined microorganisms. The filaments are light green, contain iron and are, according to Nanta,[12] the mycelia of a higher fungus. Fructification organs, too, are present, suggesting an aspergillus. This suggestion has been confirmed by cultures made from the spleens, which, in several cases, have yielded an aspergillus, in particular a sterigmatocystis (type Nanta). By injecting cultures of this fungus into rabbits, Pinoy and Nanta [13] produced an acute lethal infection, with abscesses in the kidneys and necrotic hyperemic lesions in the spleen. Nanta, therefore, called the iron nodules mycotic tubercles and spoke of a splenic mycosis. He found the fungus in 90 per cent of the cases of Algerian splenomegaly.

Shortly after the publication of the papers by Nanta and his associates, Emile-Weil, Gregoire and Flandrin [14] reported that the splenic mycosis is not restricted to Algiers but is also common in France. They confirmed the essential observations demonstrating the fungus in seven of sixteen cases of splenic anemia. The spleens were as large as they are in leukemia and showed the brownish nodules in varying numbers. In the nodules, mycelia, organs of fructification and large spores were seen surrounded by much dense connective tissue. Many foreign body giant cells were present. The clinical picture of these cases was that

10. Gamna, C.: Haematologica **4**:129, 1923, and **5**:271, 1924; Arch. per le sc. med. **49**:109, 1927.

11. Nanta, A.; Pinoy, E., and Gruny, E.: Compt. rend. Soc. de biol. **94**:635, 1926.

12. Nanta, A.: Ann. d'anat. path. **4**:573, 1927.

13. Pinoy, E., and Nanta, A.: Compt. rend. Soc. de biol. **97**:67, 1927.

14. Emile-Weil, P.; Gregoire, R., and Flandrin, P.: Ann. d'anat. path. **4**:587, 1927; Bull. et mém. Soc. méd. d. hôp. de Paris **43**:713, 1927.

of hemolytic jaundice or of Banti's disease. Most of the patients showed a tendency to bleed. There were five males and two females. The age in five cases was between 15 and 24 years and in two cases about 50.

Askanazy [15] reported that he had once seen mycelia-like structures in the smears obtained from the spleen of an anemic infant. This observation was brought back to his mind when he studied, together with Schweizer,[16] the spleens of five patients with Egyptian splenomegaly. These spleens, too, often showed the siderotic nodules with the mycelia of a fungus. He and Weil used the term "mycotic splenomegaly."

An etiologic explanation of the obscure cases of splenic anemia is of great importance and calls for an immediate reinvestigation. It will be shown in this report that the observations of the French and Swiss authors are correct with regard to the occurrence of higher fungi in the spleen in human beings. It is doubtful, however, whether one is justified in speaking of a disease entity, of a mycotic splenomegaly which leads to anemia, because the fungi apparently are found in various pathologic conditions, and patients with splenic anemia but no fungi in the spleen are seen, in whom the condition cannot be distinguished from those cases with positive evidence.

CASES OF SPLENIC ANEMIA IN ADOLESCENCE

During the last year we had the opportunity to study three spleens which had been removed from three white young men with a clinical picture similar in many respects to that described by Nanta, Pinoy and Gruny and by Emile-Weil, Gregoire and Flandrin. Hemorrhagic diathesis, however, was not observed. Dr. Birch will discuss the clinical aspect of these cases elsewhere.

Two of the patients were 14 and one was 15 years of age. The family histories contained nothing of importance. The three boys were born in the middle western part of the United States, where they had spent their lives, except one who had been in Italy for six months. They had passed through the common infectious diseases of childhood such as measles, scarlet fever, whooping cough and chickenpox. The symptoms dated back for from three months to five years. Two of the patients had repeated attacks of severe abdominal pains accompanied by vomiting, fever and headache. One of the boys reported that at the time of the attacks his face was a peculiar reddish brown. In the third case the symptoms had developed gradually, consisting of progressing weakness, shortness of the breath after exertion and sharp pains in the upper part of the abdomen. The clinical examination showed the boys to be pale and poorly nourished, with large abdomens. Swelling was seen in the lymph glands of the neck, axilla and groin. The liver was palpable, its lower margin being felt from 2 to 4 fingerbreadths below the costal margin. The spleens were large and hard. Of the laboratory

15. Askanazy, M., and Schweizer, A.: Schweiz. med. Wchnschr. **57**:777, 1927.

16. Schweizer, A.: Schweiz. med. Wchnschr. **57**:1017, 1927.

evidence, a negative Wassermann reaction, normal coagulation time of the blood and normal fragility of the erythrocytes were of special note. The bilirubin content of the serum was distinctly increased. The erythrocytes numbered about 3,700,000. There was a more or less pronounced leukopenia (from 3,000 to 6,000 white cells per cubic centimeter. In differential counts, from 42 to 54 per cent of the cells were found to be neutrophil granulated leukocytes, from 39 to 51 per cent lymphocytes and from 2 to 3 per cent monocytes. In two cases, eosinophil leukocytes were absent, while in the third case they mounted to 7 per cent. All three patients seem to have been benefited by the splenectomy.

The spleens will be described separately, because they show important differences.

Case 1.—*Spleen.*—The organ weighed 278 Gm. and measured 11½ by 3 by 9½ cm. The capsule was thickened and the surface was grayish red. The consistency was that of the normal organ. On cutting, the pulp was found to be deep grayish red. The follicles were distinct, round, grayish-white nodules, about 1 mm. in diameter. They were surrounded by a dark red line. The trabecles were seen as fine gray lines. Some of them contained small, reddish-brown patches.

In microscopic sections, the sinuses stood out distinctly and were filled with erythrocytes, lymphoid cells and granulocytes. The sinuses about the follicles were dilated and packed with red cells. The lining endothelium was swollen and bulged into the lumen. In the cords the reticulum was not increased. There were numerous cells with an indistinct cytoplasm and oval nuclei that contained a scanty chromatin net. Between these cells were found erythrocytes, many eosinophil leukocytes and a few myelocytes, with an oxyphilic granulation.

The follicles were large and were composed of three distinct layers. There was a large germinal center of lymphoblastic type, an intermediary zone of small lymphocytes and an outer zone of larger lymphoid cells. In this zone erythrocytes were seen lying free between the other cells. A few of the follicles were almost completely destroyed by hemorrhages.

The trabecles were of normal thickness. Many of them contained hemorrhages, and the free red cells pushed apart the bundles of connective tissue fibers and the elastic fibers. Most of the erythrocytes were well preserved. Between them were a small number of flat and branched cells filled with iron granules. There were many newly formed, narrow capillaries, with a distinct endothelium which crossed the hemorrhagic areas.

The arteries appeared unchanged. The veins were dilated, and the hemorrhages were found in their vicinity. The endothelium of the veins often was swollen and vacuolated. The neuclei stained pale.

Liver (piece from the lower margin of the left lobe).—Thin strands of fibrillar connective tissue extended from the periportal septums, which were thickened. These strands partly encircled the acini. The liver cells were filled with glycogen. Many of them had two or three nuclei. The portal capillaries were narrow and the Kupffer cells were enlarged. There were areas in which the structure of the liver was greatly altered. Irregular groups of liver cells were separated by dense accumulations of polymorphonuclear leukocytes. In these accumulations, liver cells with a narrow rim of cytoplasm could be seen. The groups of liver cells did not show central veins and there was not any arrangement of the cells to cords. Most of the liver cells were filled with pigment. There were smaller and larger, dark grayish-brown granules and homogenous, dark green casts. These casts often transversed the cells or ended near the nucleus with a nodular thickening. Around the casts there was an unstained space. Single liver cells were

large and their nuclei were pale. The intercellular bile capillaries were filled with dark green material, but they were not much distended. The portal capillaries were narrow, and the Kupffer cells contained dark brown pigment.

In this case, cultures on Sabouraud agar were made from the spleen immediately after the operation. They remained sterile for over six weeks.

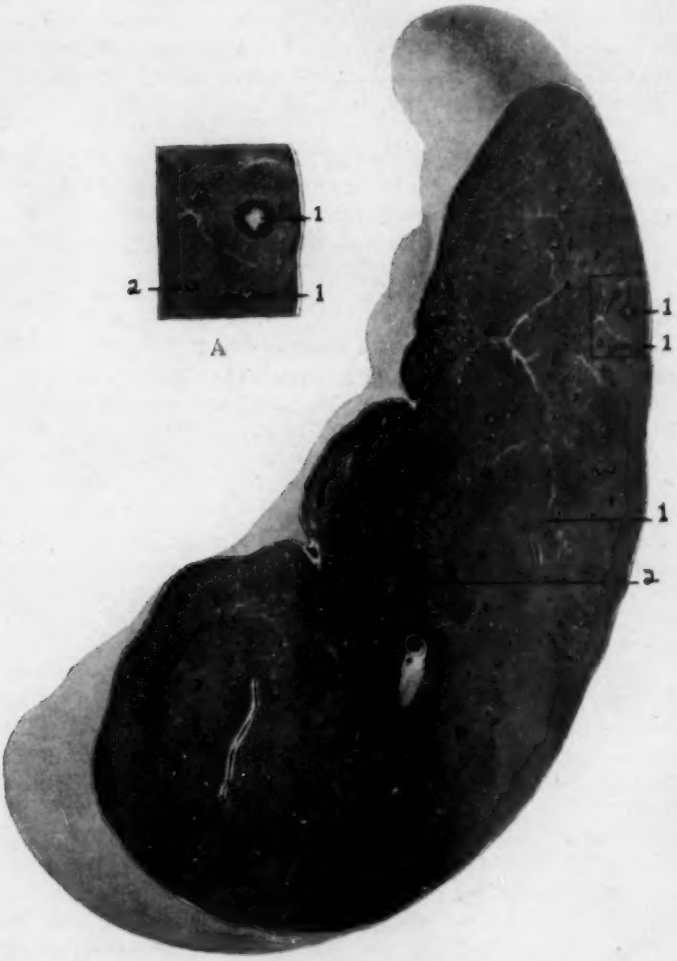

Fig. 1 (case 2).—Spleen of patient with juvenile splenic anemia. *A* indicates enlargement of outlined section of spleen; 1, iron nodules connected with trabecles; 2, follicles, both surrounded by hyperemic-hemorrhagic zones. The difference in the color between the nodules and the follicles cannot be brought out well in a reproduction in black and white.

Case 2.—*Spleen.*—The spleen weighed 425 Gm. and its diameters were 16 by 5 by 6 cm. The surface was smooth and grayish-white, with small grayish-red depressions. It was of firm consistency. Cutting revealed a grayish-red pulp, with numerous small and slightly elevated, light yellowish-brown areas, surrounded by a dark red zone. The smallest of these areas could be seen only

with the aid of a magnifying glass, the largest had a diameter of 1 mm. These areas were most numerous near the surface. The follicles could be distinguished from the areas because their color was grayish white. They, too, showed a peripheral red zone (fig. 1).

Together with the spleen an aberrant spleen had been removed. It had a diameter of 28 mm. and was softer than the main organ. The pulp was dark red and contained a single, firm, yellowish-white nodule, 2 by 3 mm. in diameter. At the hilus of the main organ a group of firm, dark yellowish-red lymph glands was found. They measured from 7 to 20 mm.

The histologic picture of this spleen showed a marked fibrosis of the pulp and pronounced changes in the trabecles. We will describe the nodule in the aberrant spleen first because the changes there were most advanced. The nodule

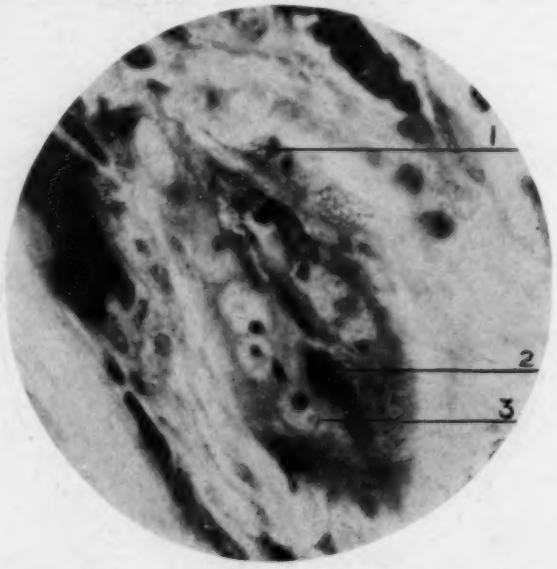

Fig. 2.—A branched thread (1) entering a foreign body giant cell (2) with many vesicular nuclei (3). Magnification, × 1,000. Fixation in Zenker-Maximow fluid; staining after Giemsa.

had a calcified center and resembled an old healed tubercle. The center was surrounded by a thick fibrous capsule with a few small nuclei. Toward the periphery the tissue became more cellular, and the bundles of collagenous tissue took the basic stain. There were groups of dark brown iron granules, free and inside of wandering cells. A few thick threads stood out distinctly. They were 4 microns thick, had a double contour and were light green. They ended with a sharp line and did not have any connection with the surrounding tissue. They gave an intensive iron reaction and were branched. In one place a branched thread was seen extending into a large giant cell with vesicular nuclei having deeply stained nucleoli (fig. 2). Here and there was found a spherical body measuring from 10 to 15 microns in diameter. It showed a highly refractive peripheral membrane. Such a body occasionally formed the end of a thread (fig. 3). The color was yellowish brown or light yellowish green. In sections stained for iron the bodies appeared a deep blue.

The nodules in the main organ revealed similar changes. There were branched and segmented threads and spherical bodies giving the iron reaction. Calcification was not observed. The pulp which surrounded the nodules was packed with erythrocytes.

From this spleen an aspergillus was obtained. It grew on Sabouraud medium in two weeks, and resembled an aspergillus fumigatus.

Lymph Glands.—These glands had wide sinuses filled with swollen and desquamated endothelial cells, some of which contained red blood cells or iron pigment. The lymphatic tissue was reduced to narrow strands in which follicles were hardly discernible. In the medullary portion plasma cells and eosinophil leukocytes were present.

Case 3.—*Spleen.*—The organ was much enlarged but had retained its normal shape. It weighed 1,315 Gm. The surface was pearl gray, with small, depressed,

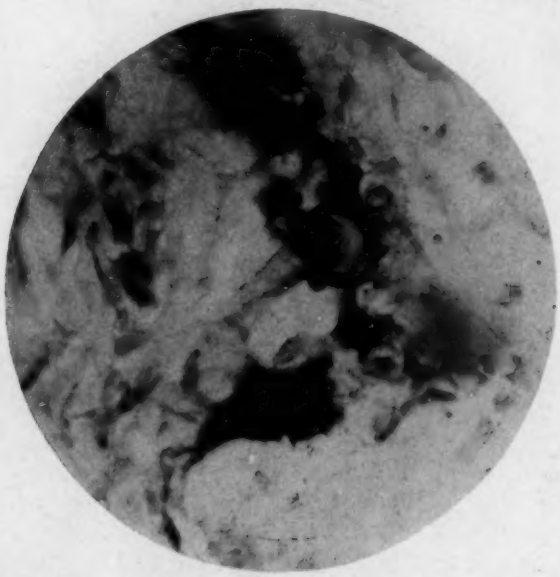

Fig. 3.—A thread terminating in a spherical body with double contoured membrane. Magnification, × 800. Technic same as in figure 2.

dark red areas. In the middle of the convex surface a deep, yellowish-brown depression, 20 mm. long and 7 mm. broad, was seen. The organ cut with difficulty and showed a cut surface which was fleshlike in appearance and dark reddish gray. Follicles could not be seen. The trabecles were distinct and from 0.5 to 1 mm. thick. Scattered over the cut surface oval, dark yellowish-brown areas were found. They measured from 0.5 to 2 mm. in diameter, and were connected with the trabecles. They were slightly elevated and felt harder than the pulp. On a section made through the largest longitudinal diameter, sixteen such areas were counted. They were most numerous near the capsules. An incision made through the depression on the surface showed a deep red hemorrhage into the pulp, with several confluent, deep reddish-brown and grayish-white patches.

There was an aberrant spleen which was softer than the main organ and showed distinct malpighian bodies. The yellowish-brown nodules were not found. There was a group of enlarged and dark reddish-gray lymph glands.

Microscopic examination of the pulp showed a marked fibrosis which was most pronounced near the capsule and the trabecles. The cords contained accumulations of plasma cells and eosinophil and neutrophil leukocytes and myelocytes. The granulocytes were most numerous in the vicinity of the malpighian bodies. Eosinophil leukocytes and myelocytes predominated. The malpighian bodies were large and showed a differentiation into three layers.

The yellowish-brown areas consisted of a dense, fibrillar, basement substance. Some of the fibrils, especially in the periphery, stained deeply with basic dyes. The elastic fibers were often fused together and formed thick, tortuous bands. Many of these bands and bundles were covered with fine, dustlike, iron granules. There were large deposits of deep brown iron granules and of granules which were lighter brown and did not give the iron reaction. Interlaced with the collagenous fibers one found peculiar trabecular structures which were not con-

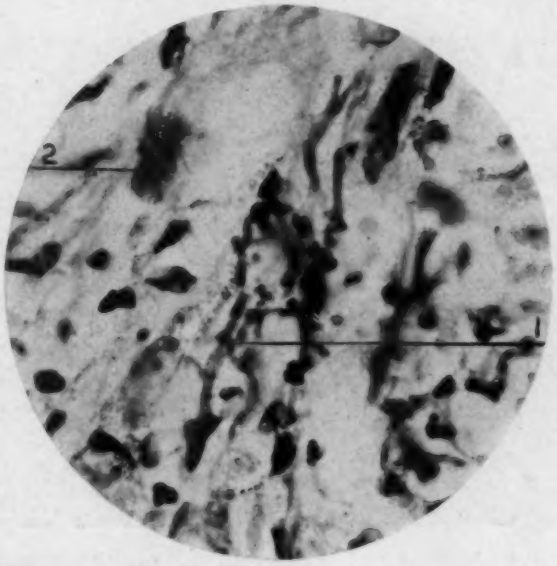

Fig. 4.—1 indicates branched mycelium; 2, granules of hemosiderin. Magnification, × 800. Technic same as in figure 2.

nected with them. In preparations stained with hemalaun-eosin they appeared light yellowish green which in places passed over into deep blue. In Giemsa sections the picture was colorful, the trabecles appearing in different shades from light yellowish green to deep sky blue. Where the trabecles lay loosely they revealed lateral branches (fig. 4). Their thickness amounted to 4 microns. Many trabecles showed a double contoured membrane which was more refractive than the central portions. There was a distinct segmentation. In the peripheral parts of the nodules were found thin, branched filaments which extended into the surrounding pulp. They were only 2 microns thick, and gave a positive iron reaction, as did the thicker threads. There were also many of the spherical bodies which were described in the first case. Threads as well as bodies were often seen inside of huge foreign body giant cells.

The arteries which were located in the iron nodules had a thickened intima. Their elastica interna was broken up into several layers and covered with iron

granules. In the media branched, light green trabecles were present which formed a wreath around the lumen and sent branches toward the lumen and toward the periphery. The pulp adjacent to the nodules was rich in red blood cells.

Many trabecles contained hemorrhages. There were numerous flat macrophages loaded with iron, and newly formed capillaries, but the light green trabecles were not seen. The hemorrhages were located near dilated veins with a swollen and partly desquamated endothelium. The arteries appeared unchanged.

SIDEROSCLEROTIC NODULES IN THE SPLEEN IN PATIENTS WITH SICKLE CELL ANEMIA

Nodules identical in their macroscopic and microscopic appearance with those just described were found in three cases of sickle cell anemia in colored boys, aged 6, 8 and 16 years. These cases have been reported

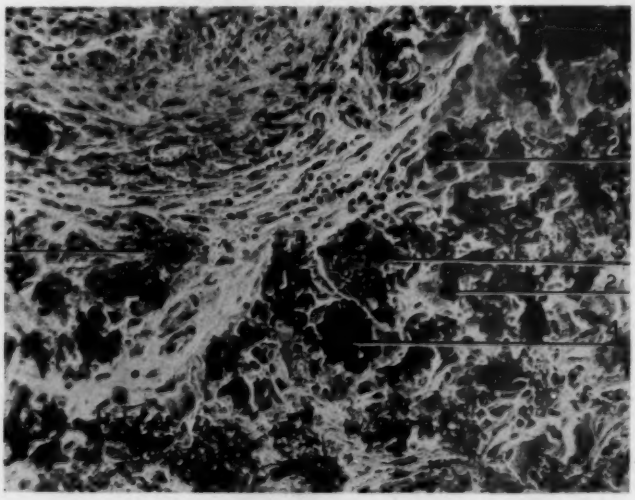

Fig. 5.—Siderotic nodule in spleen of patient with sickle cell anemia; 1 indicates mycelium; 2, structures resembling fructification organs; 3, foreign body giant cells; 4, calcified mass without structure. Magnification, × 400. Technic same as in figure 2.

elsewhere. The two younger boys died from tuberculosis, while the third patient succumbed to a severe icterus, following an injection of arsphenamine for secondary syphilis. The spleens contained the brown nodules with many light yellowish-green, branched and segmented trabecles and thinner filaments. The spherical bodies, too, were present. Large multinucleated protoplasmic masses surrounded these structures (fig. 5).

In these cases, however, the spleen was not at all, or only a little, enlarged. In the syphilitic boy it weighed 150 Gm., while in the two tuberculous children it weighed only 10 Gm. In the later cases it contained tubercles.

SIDEROSCLEROTIC NODULES IN A TUBERCULOUS SPLEEN

Several years ago one of us (R. H. J.) performed an autopsy on a white man, aged 37, who had died from tuberculous meningitis. There was a severe tuberculosis of the urinary tract and a diffuse caseation of both suprarenals. There was a solitary tubercle in the cerebellum. The spleen weighed 750 Gm. and contained, besides, numerous caseated tubercles, a few opaque and yellowish-white areas the size of a pinpoint. The microscopic picture was puzzling, and the sections were filed away for later study. Comparison of this case with the cases of splenic anemia and sickle cell anemia revealed that the underlying changes were almost identical, the only difference being that the thick threads were calcified and showed a less intensive iron reaction. There were single, foreign body giant cells containing the spherical bodies.

COMMENT

Are the yellowish-green, branched and segmented threads and the spherical bodies in the iron nodules of the spleen mycelia of an aspergillus, with their fructification organs, as emphasized by Nanta and Weil and their associates, or are they peculiar forms of an iron pigment as have been described by the majority of the earlier investigators? The most important fact favoring the French investigators' conception is the positive cultures. It seems, however, that different types of *Aspergillus* may lead to the formation of the iron nodules. While Nanta and Pinoy obtained a fungus which belongs to the sterigmatocystis nidulans, type Nanta, Weil, Chevallier and Flandrin described *Eurotium amstelodami.* The fungus cultivated in one of our cases resembled *Aspergillus fumigatus.* Weil said that the structures in the sections are so typical that they do not leave any doubt about their nature, even in cases in which the cultures have been negative. Indeed, the branched and segmented threads with the double contour and the even thickness resemble a mycelium more than anything else. The finer filaments in the periphery of the nodules often can be followed over a long distance, and their branching is most striking. The reason why the mycelia have not been recognized as such by previous investigators is apparently the difficulty to stain them in sections as long as they are not covered with iron or calcium. The later changes may obscure their structure. For demonstrating the fungus in sections we recommend the Giemsa staining of Zenker fixed material. Most of the threads appear bright blue on a purple background, and even the parts which do not take the stain stand out distinctly.

The identification of spherical bodies with fructification organs is more difficult. In our cases, the bodies did not have the phialides and the crowns of gonidia which one is accustomed to see in the cultures. There are two possibilities. Either the heads of the fungus do not

develop gonidia when growing in tissues or the latter have been destroyed in the course of regressive changes, since the heads are often covered with layers of iron and calcium salts. In aspergillosis of the lung, typical fructification organs are found, which, however, are absent in the rare cases of aspergillosis of the stomach. That the spherical bodies in the iron nodules belong to the fungus is indicated by the fact that the threads often terminate into them. The possibility that some of the bodies are huge spores, as suggested by Weil, also must be considered, but this question cannot be answered from the picture seen in microscopic sections.

The iron nodules containing the fungi develop from intratrabecular hemorrhages. Nanta thought they were derived from the follicles. Hemorrhages into the follicles occur, but they are rare compared to those in the trabecles. Kraus explained the intratrabecular hemorrhages by a destruction of the wall of the veins, while Christeller and Puskepellis emphasized degenerative changes of the arteries, and Eppinger spoke of rupture of the blood vessels. Hennings was not able to find such ruptures, nor did we. The degenerative changes of the arteries are present only in the fully developed nodules, while they are absent from the recent hemorrhages. It seems more likely, therefore, that they are secondary to an invasion of the wall of the arteries by the fungus. The extravasated blood is found around the veins in the peripheral parts of the trabecles. The veins of the spleen do not have a wall of their own. They are endothelial lined tubes in the trabecles (Schaffer). Degenerative changes of their endothelium, together with an increase of the intravenous pressure, will be sufficient to permit red cells to ooze out from the lumen into the surrounding tissue. The increase of the intravenous pressure is indicated by the engorgement of the pulp with blood and by the dilatation of the veins. Degenerative changes of the endothelium of the veins were observed repeatedly in our cases.

The extravasated blood cells break down and iron pigment is taken up by histiocytes. There is not any difference from the picture seen in hemorrhages in other organs. The hemorrhages are transformed to the yellowish-brown nodules when an aspergillus settles in this area. A mycelium develops, and it absorbs the iron liberated from the erythrocytes, as do the connective tissue fibers and the elastic membranes. Thus, the mycelium assumes a yellowish-green color and gives the microchemical reactions from which Kraus concluded that the threads are deposits of iron phosphates. Branches of the mycelium may invade the adjacent pulp and may break into the wall of the arteries, the intima of which becomes thickened. Fructification organs develop, but according to our opinion, remain rudimentary.

Compared to the pathologic changes produced by the aspergillus in other organs there is little reaction to the fungus in the trabecles of the spleen. The connective tissue proliferates and later becomes sclerotic. There are a few cellular accumulations composed of lymphocytes, eosinophil cells, mast cells and plasma cells. Giant cells appear often, forming huge protoplasmatic masses with many vesicular nuclei which have distinct nucleoli. They engulf the mycelium and the fructification organs or their remnants. The iron incrustation later is followed by calcification, and fungus and sclerosed connective tissue finally may fuse together to a structureless mass, the origin of which can no longer be recognized.

Apart from the siderotic nodules, the spleens do not show characteristic changes. There is a fibrosis of the pulp, while the follicles remain intact. There is an important difference from the Banti spleen in which, according to Banti himself, the fibrosis starts in the follicles. There are small areas of granulopoiesis in the pulp. Single normoblasts and bone marrow giant cells may be found.

It has already been said that the brown nodules are not typical of a certain disease, but that they have been observed in different pathologic conditions. The exactness with which they have been described and depicted by the earlier authors varies greatly, but there can be little doubt that identical structures have been seen in leukemia, Hodgkin's disease, syphilis, hereditary hemolytic jaundice, cirrhosis of the liver, old anemic infarcts, etc. In addition, we found them in splenic anemia, in sickle cell anemia and in tuberculosis of the spleen. Nanta, too, mentions that the fungus occasionally is found in hemolytic jaundice, Banti's disease and syphilis.

The occurrence of the mycotic nodules in hemolytic jaundice and in sickle cell anemia, both of which conditions are hereditary, points toward the possibility that there are certain predisposing factors which make the spleen a suitable culture medium for the aspergillus. Familiary hemolytic jaundice, sickle cell anemia and many of the other diseases in which the siderotic areas have been seen have one thing in common, namely, the engorgement of the splenic pulp with blood cells. This accumulation of the erythrocytes in the spleen perhaps leads to a change of the tissue reaction, which is important since it is known that the fungi grow only in acid mediums.

The French authors say little about the mode of infection with the aspergillus. The possibilities for the gonidia of the fungus getting into the body are many. Nanta mentions ulcerations of the leg and a marked bronchitis in his patients. The frequency of ulcers of the leg has been repeatedly emphasized in sickle cell anemia. There is an increasing interest in mycotic bronchitis not only in tropical and subtropical regions but also in the temperate zones. In the history of our patients nothing is

said about ulcerations or bronchitis. There were three cases of severe tuberculosis. The second patient in the group of splenic anemias had suffered from a blood poisoning following an injury. In future cases special attention should be given to the possible portal of entrance of the fungus.

According to Weil, Chevallier and Flandrin's [17] most recent report, the splenic mycosis can be recognized before the removal and examination of the spleen with the aid of a complement fixation reaction, a suspension of the gonidia being used as antigen. This reaction, however, does not seem to be specific, because positive reactions have been obtained in leukemia and Hodgkin's disease. Or does this result indicate that in these cases, too, the fungus has been present? In this connection, the question will arise as to the pathogenic properties of the fungus found in the splenic nodules. Some types of aspergillus have been described as producing histolytic substances comparable to those formed by certain strains of torula. Nothing, however, is known about a destruction of red cells by aspergillus. The storage of iron by the fungus is secondary to the breaking down of extravasated red blood cells. A similar storage of iron by a fungus is seen in the forms of madura foot which are characterized by black granules in the pus. The hemorrhages about the mycotic areas are too insignificant to lead to an anemia. Hence, for the time being, proof that the fungus causes the anemia still is lacking.

SUMMARY

1. The observation of Nanta, Weil and Askanazy that the siderotic nodules of the spleen contain mycelia and fructification organs of an aspergillus has been confirmed in two cases of juvenile splenomegalic anemia, three cases of sickle cell anemia and one case of tuberculosis of the spleen.

2. It seems that different types of aspergillus can produce similar changes.

3. The occurrence of the fungus in the spleen under different pathologic conditions does not favor the theory regarding the existence of a disease entity caused by fungus.[18] Certain changes, apparently, render the spleen suitable to an infection with *Aspergillus*. These changes consist in an engorgement of the pulp with blood and in intratrabecular hemorrhages, and are due either to disturbances in the portal circulation or to alterations of the blood.

17. Emile-Weil, P.; Chevallier, P., and Flandrin, P.: Bull. et mém. Soc. méd. d. hôp. de Paris **43**:1425, 1927.

18. Recently C. Oberling (Presse méd. **36**:2, 1928) expressed a similar idea, namely, that the infection with the fungus follows preexistent pathologic conditions of the spleen which are only a little influenced by it.

4. There is not any difference in the clinical picture between the patients with splenic anemia with, and those without, the fungi in the spleen. On the other hand, the fungi are sometimes observed in the spleen in patients without a marked anemia. Hence, there is no proof that the aspergillus causes anemia.

5. The reactions of the body against the fungus are the formation of foreign body giant cells and proliferation of the connective tissue. The mycelia and fructification organs absorb iron and calcium salts and are finally buried in sclerosed connective tissue. The latter, too, may become calcified.

6. Since the aspergillus may be present as well in enlarged as in atrophic spleens the term mycotic splenomegaly is not correct. Splenic mycosis, as suggested by Nanta, is better.

CYSTADENOMA OF THE BLADDER FROM ABERRANT PROSTATIC GLAND *

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An instructive tumor of the bladder originating from aberrant prostatic glands between the left ureteral orifice and the urethra was found at autopsy. The body was that of a well developed but poorly nourished man, aged 70, who died of lobar pneumonia.

AUTOPSY

The autopsy (T. Kon·chegg) showed the following: fibrinous pneumonia of the upper lobe of the right lung and of the lower lobe of the left lung with areas of red and gray hepatization, fresh pleurisy, marked pulmonary emphysema, chronic bronchitis, adhesions at the apexes, healing meningitis, swelling of the spleen, sagittal furrows of the liver, cystitis cystica, cystadenoma of the bladder, diverticulum of the duodenum and a scar of an old gastric ulcer.

The bladder, prostate and rectum were removed together. A soft tumor measuring 4 by 4 by 2 cm. was found under the mucous membrane of the bladder, projecting into it like a half sphere. It was located between the lower end of the left ureter and the urethral orifice and did not cause obstruction. On section, the tumor was found to be multicystic (fig. 1). Some of the cysts contained colorless, homogenous, gelatinous material. Toward the midline the tumor was not so thick, extending for 1.5 cm. with an elevation of only 0.5 cm. The mucous membrane over the tumor and immediately surrounding it was covered with tiny papules, the size of a pinhead. These papules proved to be cystitis cystica.

The prostate was the size of a horse chestnut, and of normal consistency. On section, it was grayish white.

Histologic Examination.—The bladder with the tumor and the prostate was fixed in 10 per cent formaldehyde. Sections for microscopic examination were taken from the tumor, from the sphincter of the bladder and from the prostate. These sections were dehydrated, embedded in paraffin and later stained with hemalaun and eosin and with van Gieson's connective tissue stain.

Microscopically, the tumor was located in the submucosa of the bladder and was separated from the bladder muscle beneath by an irregular band of connective tissue. In this connective tissue, there were many foci of lymphocytes and many thin-walled blood vessels filled with blood. There was no connection between the tumor and the prostate. The mucosa of the bladder showed many small cysts lined with cuboidal epithelium and surrounded by a few lymphocytes, presenting the picture of cystitis cystica.

The tumor itself contained numerous cysts lined with one or more layers of low columnar and cuboidal epithelium. The nuclei were at the bases and stained deep blue with hemalaun. In some cysts, there were many desquamated epithelial cells in a pink-staining granular substance. In other cysts, there were deep blue-staining masses, some of which were contoured and appeared like concretions of the prostate (fig. 2). In the thin layer of connective tissue between the cysts

* From the Institute of Pathological Anatomy, University of Graz, Austria.

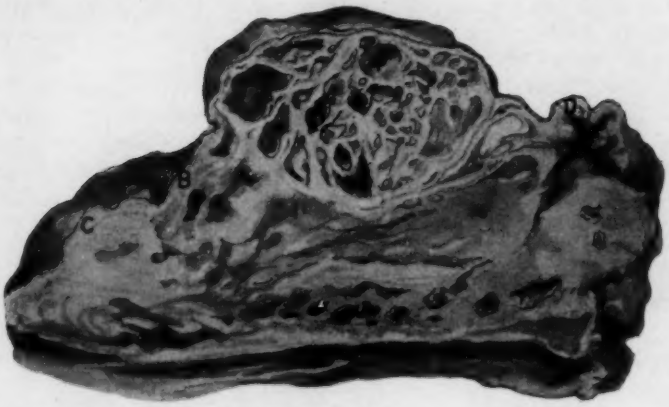

Fig. 1.—Drawing of sagittal section of cystadenoma of the bladder: *a*, indicates the cystadenoma; *b*, the sphincter muscle of the bladder; *c*, the prostate and *d*, the mucosa of the bladder.

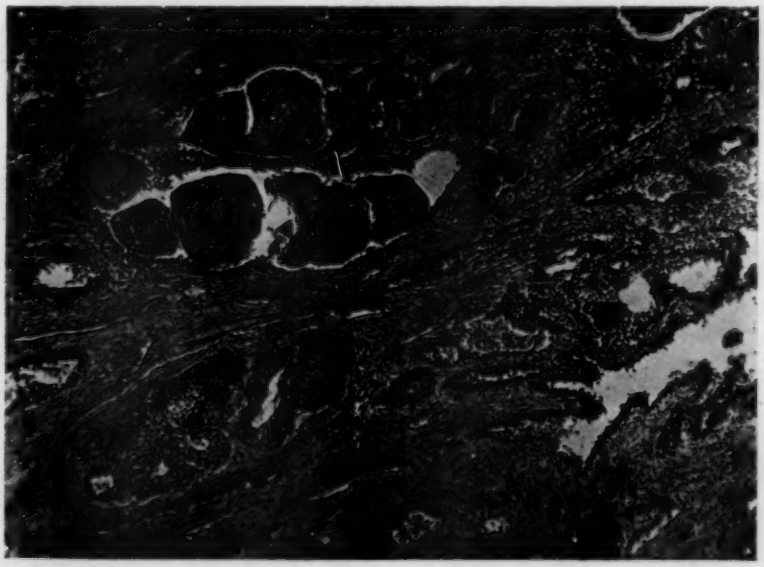

Fig. 2.—Microscopic photograph of cystadenoma of the bladder; showing the prostate-like glands with cellular débris and contoured concretions.

a few muscle fibers were shown staining a dirty yellow by the van Gieson stain. A few foci of lymphocytes and a few small blood vessels were also found in the connective tissue between the glands. There was no proliferation of the epithelial cells through the basement membrane. The cysts did not contain the numerous papillary projections found in the prostate.

One section contained a piece of the tumor, bladder sphincter and prostate together (fig. 1). No connection was found microscopically between the tumor and the prostate. The prostate was made up of epithelial lined cysts with numerous papillary projections. The epithelial lining was low columnar and cuboidal, and the nuclei were at the bases of the cells. The glands of the prostate contained numerous corpora amylacea. The interglandular connective tissue contained numerous muscle fibers and small blood vessels.

The microscopic diagnosis was cystadenoma of the bladder, cystitis cystica and hypertrophy of the prostate.

Such a tumor of the bladder containing epithelial lined cysts with contoured concretions, also interglandular smooth muscle fibers, would indicate a growth originating from prostatic glands. Since there was no connection either grossly or microscopically between the tumor and the prostate, it would further indicate that the tumor originated from aberrant prostatic glands. Aberrant prostatic glands in the lower part of the posterior wall and the neck of the bladder have been found by several authors.

COMMENT

In 1913, Herxheimer [1] reviewed the literature on this subject and found that the most common sites of aberrant prostatic glands are the upper part of the urethra and the neck of the bladder. Most authors agree that the tumors of the bladder in the cases that they reported originated from aberrant prostatic glands. Aschoff,[2] however, believes that cysts which he found in the bladder were from aberrant epithelial cells of that organ, and that the concretions were from necrotic cells. Cohen [1] believes that in his case consisting of small adenomas of the wall of the bladder, the tumor originated from the glands of the urine bladder proper. But it is generally agreed that there are no glands in the bladder except in cases of aberrant prostatic glands. Aschoff studied the mucosa of the bladder in several cases to determine the presence of glands as a possible source of tumors, but found only a tubular gland at the orifice of the urethra in one case.

According to Jores,[3] aberrant prostatic glands are found under the mucosa of the upper part of the urethra and in the neck of the bladder, especially in people past middle age. Aschoff found prostate-like glands under the mucosa of the bladder in the new-born as well as

1. Herxheimer, G., in Schwalbe: Die Morphologie der Missbildungen des Menschen und der Tiere, pt. 3, 1913, vol. 157, chapter 2.

2. Aschoff, L.: Virchows Arch. f. path. Anat., 1894, vol. 138.

3. Jores: Virchows Arch. f. path. Anat., 1894, vol. 135.

in adults. Jores says that these aberrant glands are not constant, and that they may undergo the same pathologic changes as the prostate. He claims that they are of prostatic origin because they are of similar structure and have similar contoured concretions. Thorel [4] found prostatic glands at the urethral orifice of the female and also between the vagina and the vulva. Virchow [4] and Aschoff also found such glands in the bladders of females. Thorel expressed the belief that prostatic glands may be found normally under the mucosa of the bladder in males, but he cannot be certain that these are aberrant glands because they maintain a connection with the prostate. Wittzack [4] and Ehrlich [4] agree with Thorel that all adenomas of the bladder are derived from aberrant prostatic glands, and that this may also be true for adenocarcinoma of the bladder.

Thorel cited four cases of tumor of the bladder. In all cases, the tumors were found between the ureters; they varied in size from that of a lentil to that of a pea, and they did not have any connection with the prostate. The microscopic structure of these tumors was similar to that of the prostate. The nuclei of the epithelial cells lining the glands were at the bases and close together, and these cells were arranged in papillae. There were also brown-staining flakes which contained concentric layers similar to the concretions in the prostate. The stroma of the connective tissue was rather thick in three of the tumors and thinner in the fourth. Thorel made a diagnosis of fibro-adenoma from aberrant prostatic glands in these cases. Similar cases were found by Klebs,[4] Klatenbach [4] and Wittzack.

Kostjurin,[5] in reporting the thirty-sixth case of fibromyoma of the sphincter of the bladder, mentions small adenomas which he believes were derived from aberrant prostatic glands.

SUMMARY

A rare case of cystadenoma of the bladder found at autopsy in a man, aged 70, who died of lobar pneumonia is reported. The tumor measured 4 by 4 by 2 cm., and projected into the bladder like a half sphere. On section, it showed numerous cysts, some of which contained a clear, gelatinous substance.

Microscopically, the tumor showed numerous cysts lined with one or more layers of low columnar epithelium with the nuclei at the bases of the cells. Some cysts contained many desquamated epithelial cells in a pink-staining, granular substance; others contained contoured masses similar to the corpora amylacea of the prostate. With the van

4. Thorel, Bruns: Beitr. z. klin. Chir. **36**:630, 1902.

5. Kostjurin, W. S.: Fibromyoma der Harn Blase, Ztschr. f. urol. Chir. **18**:197, 1925.

Gieson stain, connective tissue fibers were seen between the glands mixed with smooth muscle. There was no connection between the tumor and the prostate either grossly or microscopically. The prostate showed hypertrophy.

Some authors have found normal aberrant prostatic glands in the lower part of the posterior wall of the bladder, and other authors have found tumors originating from these glands.

The peculiar structure of the tumor described indicates a growth of prostatic origin. The lack of any connection with the prostate points to an origin from aberrant prostatic cells. It seems justifiable, therefore, to call this tumor a cystadenoma of the bladder from aberrant prostatic gland.

THE DISTRIBUTION AND AMOUNT OF CALCIUM AND OF POTASSIUM IN NORMAL TISSUE OF THE MOUSE

A HISTOCHEMICAL STUDY *

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AND

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As far as a search of the literature has shown, a complete survey of the potassium and calcium content of normal tissues has not previously been published. The results which have been obtained in such an investigation are sufficiently interesting to warrant a presentation of the gathered data.

TECHNICAL METHODS

Potassium.—The well known method of Macallum [1] has been modified so that permanent preparations can be made. Small blocks of fresh tissue (0.5 by 1 mm.) are placed for sixty minutes in a solution, the formula of which is: Solution A: In 50 cc. of distilled water to which 12.5 cc. of glacial acetic acid is added, dissolve 25 Gm. of cobalt nitrate. Solution B: In 260 cc. of distilled water dissolve 240 Gm. of sodium nitrite. For use add all of solution A to 210 cc. of solution B. Keep the solutions in the icebox. All reagents must be potassium-free.

After immersion in this mixture the fragments are washed during a period of twenty minutes in seven changes of ice cold distilled water and transferred to a solution of: glycerin or distilled water, 60 cc.; yellow ammonium sulphide, 15 cc. The fragments are left in the ammonium sulphide for thirty minutes, then repeatedly washed in changes of ice cold distilled water, and finally transferred to a 4 per cent solution of formaldehyde. After from three to twenty-four hours' fixation, they are cut by the frozen technic, dehydrated, cleared and mounted in the usual fashion.

The potash is precipitated by the cobalt reagent as yellow crystals which are blackened by the ammonium sulphide. Larger fragments must be left in the various solutions for proportionately longer periods to insure penetration of the reagents. Just how long will have to be determined by trial for the individual tissue and block size. The tissues thus prepared cannot be cut quite as thin as average routine sections. Counter stains such as hematoxylin or eosin or both may be employed but such stains mask the deposit, rendering photography particularly difficult. After fixation the tissue may also be prepared by the usual paraffin process.

Calcium.—The method employed is essentially that of Macallum. Tissue blocks are placed for from forty to sixty minutes in: sulphuric acid, 2 cc., and

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* From The Achelis Laboratory, Lenox Hill Hospital. This investigation was made under a grant from Miss J. Springer.

1. Macallum: Die Methoden der Biologische Mikroanalyse, Abderhalden, Handbuch der Biochemischen Arbeitsmethoden, Jena, Gustave Fischer, 1905, vol. 5, sec. 2, pp. 1099-1147.

95 per cent ethyl alcohol, 100 cc. They are then thoroughly washed in 95 per cent ethyl alcohol and placed in tenth-normal lead acetate solution for from thirty to forty minutes, after which they are washed for twenty minutes in distilled water and immersed for six minutes in tenth-normal nitric acid. After a thorough washing in distilled water they are put in the ammonium sulphide mixture and the routine described under potassium is followed. All that has been said in regard to potassium applies to calcium technic with equal force.

In a previous paper,[2] a method for the demonstration of sodium in tissues was described. This method was also applied in the present study, but the successful results were so inconstant that they have not been considered here in detail.

For the purpose of the present study, the fresh tissues of the normal mouse were employed. Material which has been previously fixed may on occasion give partially successful results, but in the majority of trials, the procedures described, when used with fixed tissues, result in failure.

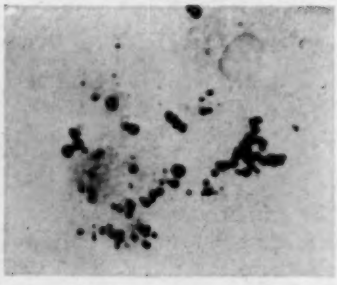

Fig. 1.—Potassium deposit in three adjacent liver cells; × 350.

A survey of our material shows that the distribution of potassium within and without the cell is fairly uniform throughout the body, irrespective of the type of cell investigated. The variations observed have to do with the relative amounts present. In the individual cells the potassium is found as a fine deposit in the cytoplasm. If these deposits are connected by a line, a fair imitation of the cytoplasmic network appears. Such an arrangement suggests that the potash is present in the knots of the network (fig. 1). Larger deposits are found in the nucleus, and the deposits are increased in this locality if the cell is in a phase of mitosis. It is further apparent that every cell is separated from its adjacent cells by a minute channel, and in this channel a fine deposit of potassium can be demonstrated. These spaces are well shown in illustrations of the uterus of the mouse, the cells of the peritoneum being pictured.

2. Rohdenburg and Krehbiel, J.: Cancer Research **7**:417 (Oct.) 1922.

Calcium, like potassium, is found without the cell in the tissue spaces as fine granules rather widely scattered. It is also occasionally observed within the cell. When observed within the cell it occurs as a fine deposit sometimes in the cytoplasm, at other times in the nucleus. One gains the impression, without, however being able to bring forth proof, that calcium is present within the cell when the cell is in its senescence, for it has not been observed in cells undergoing mitosis or in small young cells. As with potash, the amount of calcium within a given tissue may vary widely but within the cells of a given tissue there is little variation; further, such variations as are observed are possibly due to increased or decreased deposits in the tissue spaces rather than in the cells.

CONSIDERATION OF TYPES OF TISSUES

Epithelium may be roughly classified as of the stratified squamous, or of the glandular, type. In the stratified squamous type, e. g., skin, potassium deposits are not found in the keratinized layers and only small deposits in the midlayers. Larger amounts are found in the germinative layer so that the low power magnification shows the potash as a black line marking the lower layer of the epithelium. This deposit is increased about areas of regeneration such as a healing wound. The glandular type of epithelium contains much more potash than the squamous. The distribution of the potassium varies in the glandular type according to the form and function of the epithelium. These differences will be taken up in detail in the discussions on the various organs. The more physiologically active the epithelium, the greater the potash content; thus the lactating breast contains much more than the resting breast.

The calcium deposits in the epithelium occur as minute deposits in the tissue spaces and with frequency in the cells well on the way to keratinization.

Connective tissue, in contrast to epithelium, shows an almost constant potassium content which varies only when one organ or tissue contains more connective tissue than another. While in the greater part the distribution of the calcium precipitate is as has been previously described under epithelium, in some tissues the deposit occurs as long fine threads, e. g., stroma of the lymph glands, lung and brain. Those tissues in which reparative processes are frequently the site of calcific deposits show the larger amounts of calcium.

Fat shows a fine deposit of both calcium and potassium in the fibrils surrounding the fat droplets. If in a fat from one locality the stroma is denser than that taken from another, the amounts of potassium and calcium vary as does the supporting stroma.

In muscle, the potassium is deposited in largest amounts between the fibers and in smaller amounts as fine granules within the fiber itself. These fine granules are more closely packed about the nuclei. There is no difference in distribution between smooth and striated muscle, though smooth muscle may contain slightly more potassium than does striated muscle. The calcium content of muscle is distributed as small granules in the tissue spaces and occasional granules in the muscle fibers. In the tendon there are relatively large deposits of potassium, but the calcium deposition is scanty. When the tendon is markedly noncellular, both elements are only scantily deposited.

In cartilage, the potassium is diffusely deposited in small granules in the cells, and in smaller amount in the matrix. The calcium is deposited

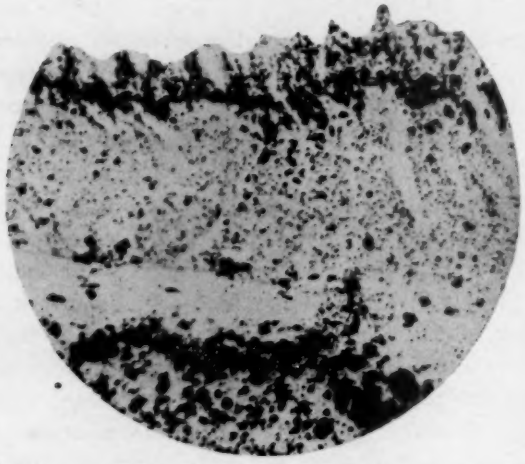

Fig. 2.—Potassium deposit in glandular portion of stomach. The heavy deposit in the upper third of the tubules should be noted. Only a portion of the muscular layer is shown; × 200.

heaviest in the matrix, where it appears as broad lines running lengthwise of the cartilage.

In bone, the potassium is diffusely deposited most markedly in the medullary canal. The calcium is deposited in the cancellous bone structure, the deposit resembling the architecture of the bone lamella.

In the formed elements of the blood, the potassium is found as minute granules in the red cells and as granules in the cytoplasm and nuclei of the white cells. Calcium was not demonstrable in either red or white cells.

Gastro-Intestinal Tract.—The potash and calcium deposits of the tongue and esophagus do not need a description since they are covered by the previous description of squamous epithelium and muscle. In the stomach at the transition from squamous to glandular epithelium, there

occurs a characteristic change in the deposition of potassium, which is deposited as fine granules in the secreting cells at their borders adjacent the gland lumen. Particularly heavy deposits are found in the outer third of each tubule, so that in a section of considerable length this region appears as an irregular black line (fig. 2). This characteristic deposit of potash continues until about the middle third of the ileum when it ceases, the outer third of the gland lumen from there on not being any different than other portions of the tubule. The heavy deposition again occurs in the sigmoid. The potassium found in the submucous and muscular layers is the same as that found in such tissues in general.

The calcium deposition does not differ from that described under muscle and epithelium.

In the salivary glands, i. e., the parotid and salivary, an unusually heavy deposition of potassium is distributed in the tissue spaces, stroma, epithelial elements and in the gland lumen. The calcium deposit in these tissues is not different from that previously described.

In the liver, fine granules of potassium are found within the cell and larger amounts in the bile passages and in the tissue spaces. At times the amount in the bile passages is sufficient to map out the biliary system. The calcium deposits occur in the stroma as small granules with occasional larger collections in the Kupffer cells and still larger amounts in the bile passages. In the gallbladder, the deposition of both potassium and calcium is the same as that in the terminal portion of the ileum, but there is much less in evidence.

The pancreas shows a heavier precipitate of potassium than does the liver. This precipitate is about equal in the cells of the islands of Langerhans and in the other secreting cells. Relatively large amounts are present in the tissue spaces, and some of the smaller ducts show large aggregates. The calcium deposit is not extensive and occurs as a fine granular deposit chiefly in the tissue spaces.

Vascular System.—The heart shows the potassium in relatively large amounts with heavier precipitate beneath the visceral pericardium and the endocardium. The calcium precipitate is scanty and is rather regularly deposited between the muscle fibers. Taking the aorta as typical of the blood vessels, one finds the potassium diffusely precipitated in the muscular coats with a scanty deposit in the intima. Calcium is deposited in a relatively large amount in the media and intima.

Respiratory System.—In the lungs, the potash is moderately thickly deposited in the alveolar epithelium, with smaller deposits in the tissue spaces and connective cells. Larger amounts are present in the bronchi, possibly because of their muscular fibers. The calcium (fig. 3) is found

as long needle-like deposits in the stroma. The mucous glands of the trachea show the usual distribution of potassium and calcium seen in other glandular structures.

Urinary Organs.—Potassium occurs in the kidney as larger deposits in the glomeruli (fig. 4), with the tubules of the Henley system next in order and the collecting tubules showing the least. There are consid-

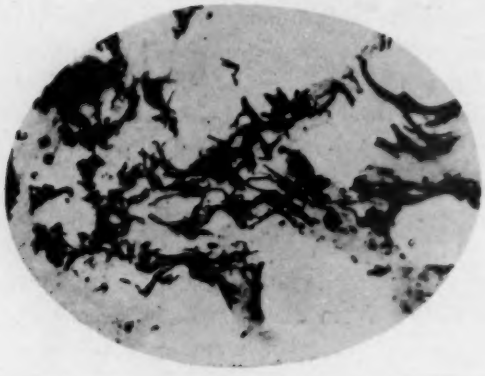

Fig. 3.—Calcium deposit in lung; × 250.

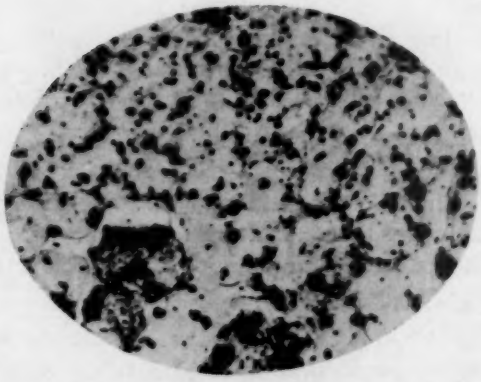

Fig. 4.—Potassium in kidney; × 200.

erable deposits between the cells and in the glomerular spaces. The distribution of calcium follows the same order but is not as extensive as one might expect, if one considers the function of the organ. The urinary bladder presents the same condition found in the muscle and squamous epithelium.

Skin.—The potash deposit in the skin has been described under squamous epithelium. The interesting feature is the distribution of this metal in the skin appendages. In sweat, sebaceous and sudoriferous

glands, potassium occurs as a fine deposit situated in the cells, the amount being dependent on the physiologic activity of the glands. In the hair follicles (fig. 5), considerable amounts are found in the bulb of the follicle. The calcium deposition in the skin occurs as a fine granular deposit chiefly in the tissue spaces and in the superficial layers of the epithelium.

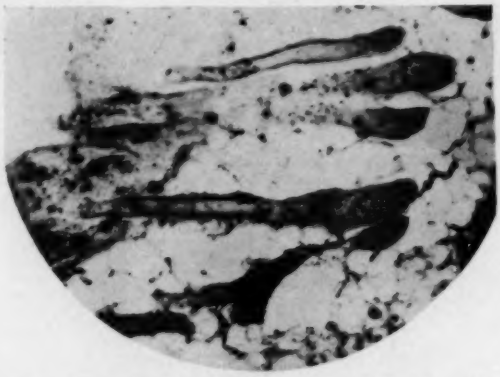

Fig. 5.—Potassium in hair follicles. The deposit of potash in the adjacent fat should be noted; × 200.

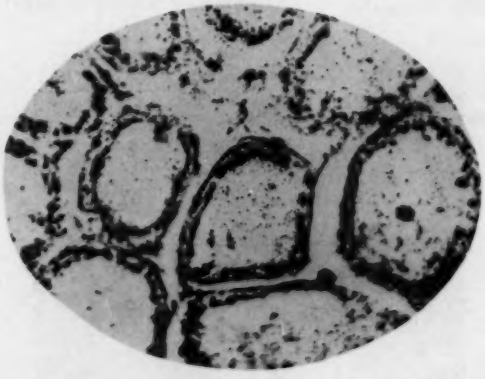

Fig. 6.—Potassium in testes. The almost complete absence of deposit in the lumen of the tubules should be noted; × 200.

Genital Organs.—In the testes, potassium is deposited in fairly large granules in the cells of the semeniferous tubules and in masses almost as large in the tissue spaces (fig. 6). Potassium was not demonstrable in the head of the spermatozoa, although occasional granules were encountered in the tail. The calcium in the testes occurs as fine granules in the tissue spaces and in the lumen of the semeniferous tubules. The heads of the spermatozoa gave the reaction for calcium (fig. 7).

In the seminal vesicles and prostate, the potash and calcium deposits were the same as those in other glandular organs.

In the ovaries, potash occurred as a fine deposit throughout the organ, the amounts being smallest in the receding corpora lutea and albicantia. In the graaffian follicle, the granulosa layer contained potash as fine granules, while the ovum was a solid mass of potassium (fig. 8). The calcium of the ovary was distributed in fine granules in the tissue spaces and was largest in amount in the corpora albicantia.

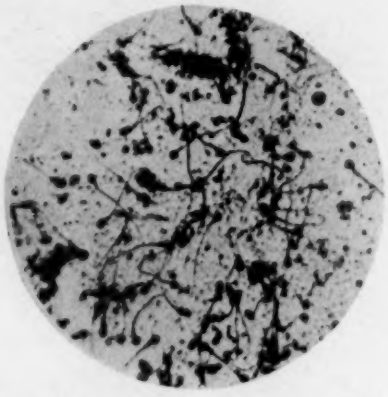

Fig. 7.—Spermatozoon, showing calcium deposit in head; × 350.

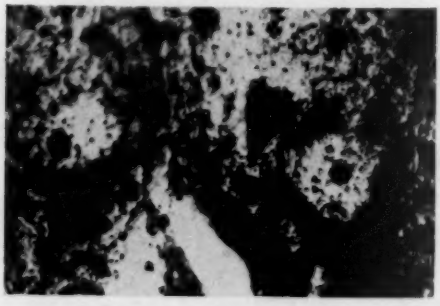

Fig. 8.—Potassium deposit in graaffian follicles showing ovum a solid deposit of potash; × 300.

In the uterus, the precipitate of both the minerals in question was the same as that found in other muscular organs lined with epithelium. In one of the sections of the uterus, the pavement nature of the peritoneal covering of the organ was well shown, each cell being separated from its neighbor by a fine potassium deposit.

In the breast, the potassium was found as fine granules in the cells, the deposit being heaviest at the end of the cell nearest the gland lumen.

Calcium occurred more frequently than in most tissues, but, as in other areas, was found chiefly in the tissue spaces.

Lymphoid System.—Potassium occurred in the lymph nodes as a diffuse fine to a slightly coarse precipitate in both lymph cell and tissue spaces. It was slightly heavier in the germinal centers than elsewhere in the gland. The calcium deposits were chiefly in the tissue spaces, but occasionally stroma fibers were found in which there was a heavy calcium deposit. The same statements hold good for the spleen.

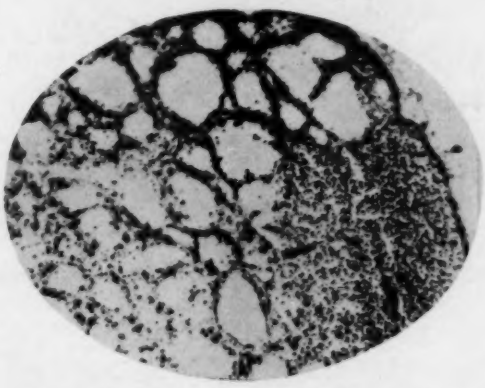

Fig. 9.—Thyroid and embedded parathyroid showing potassium deposit; × 200.

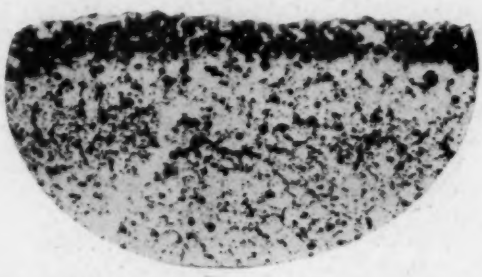

Fig. 10.—Potassium in suprarenal. The density of the deposit in the peripheral zone should be noted; × 200.

Endocrine System.—In the thyroid and parathyroid glands (fig. 9), the potassium is distributed evenly as granules of moderate size in the cells of both the secreting epithelium and the stroma. The calcium occurs as small granules in the tissue spaces and occasionally in the gland alveoli.

In the pituitary gland, the potassium is distributed as fine and coarse granules in all three portions of the gland. The calcium is deposited

as small granules sometimes within the cell but more often in the cellular spaces.

In the suprarenal gland (fig. 10), the potassium deposit occurs as a heavy ring in the outer zone of the gland and as fine deposition in the medulla and cortex. A similar concentration also occurs with calcium, the outer zone showing a dense deposit; the other zones show a scattered fine deposit, chiefly in the tissue spaces.

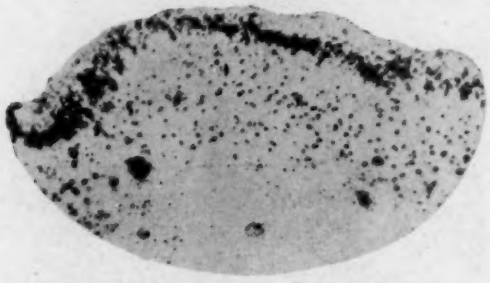

Fig. 11.—Potassium in frontal lobe of brain; × 200.

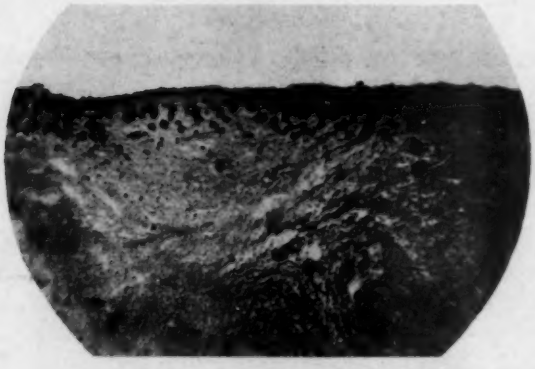

Fig. 12.—Calcium in frontal lobe; × 200.

The thymus shows the heaviest deposit of potash encountered in any of the endocrine glands. Every cell shows a heavy deposit, and such deposits are particularly heavy in the corpuscles. The calcium content is slight and occurs as fine granules in the tissue spaces.

Nervous System.—The potassium deposit occurs in largest amount in the gray matter of the frontal lobes (fig. 11), to a lesser degree in the motor area and in least amount in the posterior lobes. In the hind brain the deposit is about as heavy as in the motor area. Potassium is not demonstrable in the white matter. The calcium deposit in the frontal and posterior lobes occurs as a single zone in which the deposition

is heavy. This zone occurs in the outer portion of the gray matter (fig. 12). Occasional deposits of calcium are encountered in the isolated cells beneath the well demarcated zone. In the motor area (fig. 13), the deposition of calcium occurs in two zones. One, the outer zone, is a continuation of the deposit described; the other zone lies about midway in the gray matter and is wider than the zone at the

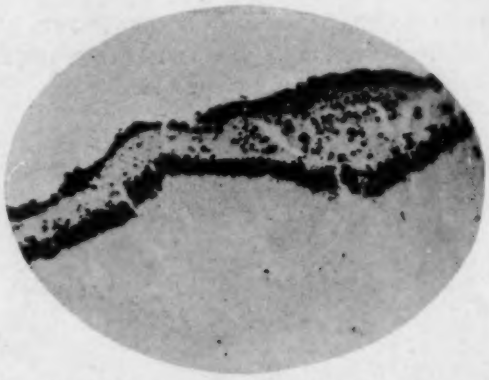

Fig. 13.—Calcium in motor area of brain; × 200.

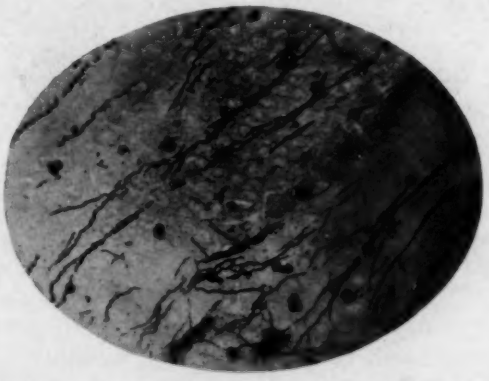

Fig. 14.—Fibers in motor area of brain showing calcium deposit; × 200.

periphery. In addition to this, isolated fibers are demonstrable in the white matter of the motor area in which a heavy deposition is found (fig. 14). In the hind brain, zones of calcium deposit are not found. The metal occurs as fine scattered granules in the entire area.

In the spinal cord, the potassium occurs as fine granules in the gray matter and all the supporting structures of the columns. Potash is not demonstrable in the cells of the anterior or posterior horn (fig. 15). The cells lining the central canal show heavy potash deposits. The calcium deposition in the cord is in the form of a heavy deposit

two layers deep, one at the periphery of the cord, the other at the edge of the gray matter. In the peripheral nerves there is a fine potassium deposit in the nerve sheath and scattered granules within the nerve itself. Calcium is found only as scattered granules.

COMMENT

It is obvious that statements as to the significance of the potassium and calcium content and distribution in normal tissues are not possible on the basis of the present study. The results, however, are suggestive both for further study and present speculation.

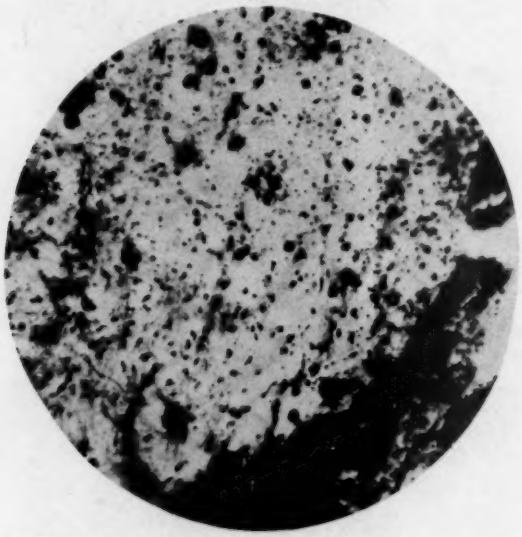

Fig. 15.—Potassium in spinal cord. The absence of deposit in cells of the gray matter should be noted; × 200.

It is evident that potassium is found in largest amounts in tissues in which physiologic activity is marked, e. g., pancreas, salivary glands, liver, etc. Areas in which cell replacement is continually going on, e. g., the layer germinativa of the skin, are also the site of a heavy potassium deposition. It is also evident that calcium is found in greatest amounts where reparative processes are most often associated with calcification, e. g., lung, lymph glands.

In addition to these general observations, there are specific points as to the possible importance and significance of which one can only speculate. To list these in order one might commence to theorize as to the significance of potassium in the female ovum while calcium is found in the male spermatozoa. Another question is the significance of the segregation of potassium and calcium in the peripheral zone

of the suprarenal gland, and possibly correlated is the explanation of the dense potassium deposit in the thymus. Another most interesting series of speculations might be made on the basis of the peculiar calcium and potassium concentrations in the central nervous system. To these and a number of other pertinent queries we cannot give an answer at the present. We believe that for the present it is sufficient to place the observations on record without comment.

SUMMARY

A histochemical examination of the normal tissues of the mouse has been made to determine the potassium and calcium content and its location within the tissue. It has been shown that potassium is found in largest amounts in tissues in which there is physiologic activity, and in localities in which repair and regenerative processes are taking place. Peculiar concentrations of potassium and calcium have been found in the nervous system and other places which for the present cannot be connected with the functions of the tissues involved.

PRIMARY CARCINOMA OF THE LIVER

REPORT OF THREE CASES *

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Primary carcinoma of the liver is an interesting pathologic entity of rare occurrence. Despite the infrequency of this condition there are numerous articles on the subject, and one gains the impression that an unusually high percentage of all diagnosed cases is reported.

In 1901, Eggel [1] made a complete review of the medical literature and accepted 163 cases to which he added one of his own. Table 1 compiled from the records of various medical centers shows the uncommon occurrence of primary carcinoma of the liver.

Of the 29,215 necropsies, only thirty-nine revealed primary carcinoma of the liver, an average percentage of 0.133. Winternitz [2] in 1916, collected statistics which showed the percentage to vary from 0.028 to 0.3. At the hospital of the State University of Iowa, the records show two cases, or 0.13 per cent, in 1,500 consecutive postmortem examinations during the last fifteen years. A third case occurred in a patient who is living, so it cannot be included in the necropsy figures. The rarity of the condition and a recent unusual case stimulated this report.

Primary carcinoma of the liver differs from most carcinomas in that it occurs in two distinct age groups: those of the usual carcinoma age (from 40 to 60), and those of infancy and childhood. A relatively large proportion of the cases are included in the latter group. Griffith [3] has made the most complete report of primary carcinoma of the liver in childhood. He collected from the literature twelve cases, included the cases of Steffen and of Castle (twenty-four and twenty, respectively) and added one of his own, making a total of fifty-seven cases occurring during the first sixteen years of life. Of the fifty-five cases in which the age was given, twenty-four or 44 per cent, occurred in the first two

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1. Eggel, H.: Ueber das primäre Karzinom der Leber, Beitr. z. path. Anat. u. z. allg. Pathol. **30**:506, 1901.

2. Winternitz, M. C.: Primary Carcinoma of the Liver, Johns Hopkins Hosp. Rep. **17**:143, 1916.

3. Griffith, J. P. C.: Primary Carcinoma of the Liver in Infancy and Childhood, Am. J. M. Sc. **155**:79, 1918.

years of life. Many were found at birth or within a few months afterward. It is interesting that in 106 of Eggel's patients in whom a microscopic examination was made and the age given, only four were under 16 years of age. Of the three cases in this report, two occurred at the ages of 55 and 68, respectively, and the other in a girl of 13.

The histologic classification of primary carcinomas of the liver is the most helpful and logical. In Eggel's report, he divided his cases into two great classifications: those arising from the intrahepatic bile ducts, and those arising from the parenchymal liver cell. More recently, these have been called cholangioma and hepatoma, respectively. It is our opinion that primary carcinoma of the liver and primary carcinoma of the bile duct cell type are adequate and self-explanatory nomenclatures. Writers differ as to the requirements for differentiation of these two types of neoplasms. Eggel depended largely on the morphology of the

TABLE 1.—*Occurrence of Primary Carcinoma of the Liver*

Source	Necropsies	Cases	Percentage
Guy's Hospital, London (Wheeler: Guy's Hosp Rep. **63**: 225, 1909)	5,233	15	0.287
Johns Hospkins Hospital (Winternitz: Johns Hospkins Hosp. Rep. **17**: 143, 1916)	3,700	3	0.081
Boston City Hospital (Rowen and Mallory: Am. J. Path. **1**: 677, 1925)	6,506	9	0.138
Mayo Clinic (Counseller and McIndoe: Arch. Int. Med. **37**: 363, 1926)	5,976	5	0.083
University of Minnesota (Clauson and Cabot: J. A. M. A. **80**: 909, 1923)	5,100	1	0.019
Peter Bent Brigham Hospital (Fried: Am. J. M. Sc. **168**: 211, 1924)	1,200	4	0.333
State University of Iowa	1,500	2	0.133
Total	29,215	39	0.133

cell. Both Wegelin [4] and Yamagiwa [5] laid emphasis on the type of stroma. A delicate network of capillaries and a scanty amount of fibrous tissue is characteristic of the stroma in the liver cell type, while the bile duct neoplasm has an abundant stroma. Ribbert [6] felt that intracellular bile pigment was important. Fried [7] and Winternitz independently held that three essentials were necessary for an accurate diagnosis: the type of cell (liver or duct), the arrangement of cells (cord or duct formation) and the stroma (delicate or abundant).

4. Wegelin, K.: Ueber das Adenokarcinom und Adenom der Leber, Virchows Arch. f. path. Anat. **179**:95, 1905.

5. Yamagiwa, K.: Zur Kentnis des primären parenchymatosen Leberkarzinoms, Virchows Arch. f. path. Anat. **206**:437, 1911.

6. Ribbert, H.: Das maligne Adenom der Leber, Deutsche med. Wchnschr. **35**:1607, 1909.

7. Fried, B. M.: Primary Carcinoma of Liver, Am. J. M. Sc. **168**:241, 1924.

8. Wells, H. G.: Primary Carcinoma of the Liver, Am. J. M. Sc. **126**:403, 1903.

Wells [8] reported a case in 1903 in which both hepatic and bile duct epithelium were observed. He believed that the bile duct epithelium and the parenchyma cells are embryologically one. He argued that in a malignant tumor in which reversion to the embryonal condition is a most important feature, it is natural to find the two types of cells present. "There is no doubt that embryologically liver cells and bile ducts have a common origin, but we believe that in the fully differentiated organism, cells which were originally totipotent, become and remain unipotent. In short, bile ducts produce bile ducts and carcinoma of bile ducts, while liver cells are capable of reproducing themselves to the point of malignancy." [9] The accuracy of applied pathology speaks for the cell's maintaining its characteristics when it is completely differentiated. However, classification of a given new growth is occasionally difficult because of a rather mixed histology. We do not believe that this characteristic is peculiar to primary neoplasms in the liver; at least, in the three cases reported in this article no such difficulty was encountered.

Vascular invasion is a striking characteristic of the liver cell type of carcinoma. Intrahepatic veins are practically always invaded. The growth may fill the vein for a considerable distance even outside the liver. Tumor thrombi extending from the liver to the right heart are described. Extending in a retrograde manner in the portal vein the growth may reach the spleen or mesenteric veins. In Eggel's series extrahepatic tumor thrombi were found in 23 per cent of the cases. The portal vein was invaded in twenty-eight, the hepatic vein in fifteen and the vena cava in four cases.

We have compiled in tables 2, 3 and 4, eighty cases reported in the English medical literature since 1901. In this group 27.5 per cent showed extension into the extrahepatic vessels: the portal vein was invaded in twenty, the hepatic vein in nine and the vena cava in five cases.

Despite the frequent and extensive vascular involvement and the extreme cellularity of the growths metastases are not the rule. This is generally accepted in the literature, but since the report by Eggel, writers have been vague as to the occurrence and extent of metastases. Eggel's article reveals that extrahepatic metastases (not including the tumor thrombi) were present in 40 per cent of his patients. The glands were involved by metastases in forty of them, or 24.6 per cent of the cases. The portal and supragastric glands were involved in twenty-three, the retroperitoneal in fifteen, the thoracic in thirteen, the mesenteric in five and the clavicular in two cases. The next in order were the lungs which were involved in twenty-three cases (14 per cent), both lungs being mentioned

9. Counseller, V. S., and McIndoe, A. H.: Primary Carcinoma of the Liver, Arch. Int. Med. **37**:363 (March) 1926.

TABLE 2.—*Reported Cases of Primary Carcinoma of the Liver Cell Type from 1902 to 1926*

Number	Author and Reference	Extension into Extrahepatic Veins			Metastases						
						Lymph Nodes					
		Portal Vein	Hepatic Vein	Vena Cava	Lungs	Thoracic Duct	Mesenteric Glands	Portal Vein	Retro-peritoneum	Unusual	Bile
1	Ackland and Dudgeon: Lancet **2**: 1310, 1902	..	..	..	..	+	..	+	+	Peritoneum; omentum	..
2	Thompson: M. Press & Circ. **75**: 243, 1903	..	..	..	..	..	..	..	..	Pleura	+
3	Peabody: Tr. A. Am. Phys. **19**: 308, 1904	+	+	..	..	..	..	..	..	.....	..
4	Bays: Brit. M. J. **2**: 630, 1905	..	..	..	..	..	..	..	..	.....	..
5	Cheney: Am. Med. **10**: 21, 1905	..	..	..	Right	..	..	..	..	Suprarenal	+
6	Ewing: Proc. New York Path. Soc. **6**: 168, 1906-1907	..	..	..	..	..	..	..	..	.....	..
7	Porter: Internat. Clin. **3**: 75, 1907	..	..	..	..	..	..	..	..	Skin; spleen; kidneys; brain	..
8	Porter: Ibid.	..	..	..	..	..	..	..	..	Meninges	..
9	Muir: J. Path. & Bact. **12**: 287, 1907-1908	+	..	..	..	..	..	..	..	.....	..
10	Muir: Ibid.	+	..	..	..	..	..	..	..	.....	..
11	Muir: Ibid.	+	..	..	..	..	..	..	..	.....	..
12	Muir: Ibid.	..	..	..	+	..	..	..	..	.....	..
13	Muir: Ibid.	..	..	..	..	..	..	..	..	.....	..
14	Muir: Ibid.	+	..	..	..	..	..	+	..	Omentum	..
15	Wheeler: Guy's Hosp. Rep. **63**: 225, 1909	..	..	..	..	..	..	..	..	.....	..
16	Wheeler: Ibid.	..	..	..	..	..	..	..	..	.....	+
17	Wheeler: Ibid.	..	..	..	..	..	..	..	..	Extension to vertebra	..
18	Weber: Lancet **1**: 1066, 1910	..	..	..	Left	..	..	..	..	.....	+
19	Karsner: Arch. Int. Med. **8**: 238, 1911	+	..	..	..	..	..	..	..	.....	..
20	Karsner: Ibid.	+	..	..	..	..	..	..	..	.....	..
21	Karsner: Ibid.	..	..	..	..	..	..	..	..	.....	..
22	Karsner: Ibid.	..	..	..	..	..	..	..	..	.....	..
23	Glynn: Brit. M. J. **2**: 1192, 1911	+	..	..	..	..	..	..	..	.....	..
24	Mair: J. Path. & Bact. **16**: 389, 1911-1912	+	+	..	Both	..	..	..	..	.....	+
25	Beattie and Donaldson: J. Path. & Bact. **17**: 32, 1912-1913	..	..	..	..	..	..	..	..	.....	..
26	Castle: Surg. Gynec. Obst. **18**: 477, 1914	..	..	..	..	..	..	..	..	.....	..
27	Weber: Lancet **2**: 68, 1915	..	..	..	..	..	..	..	..	.....	..
28	Ong: Wash. M. Ann. **14**: 175, 1915	..	+	..	..	..	..	+	+	.....	..
29	Winternitz: Johns Hopkins Hosp. Rep. **17**: 143, 1916	+	..	..	Both	..	+	..	..	.....	..
30	Winternitz: Ibid.	+	..	..	Both	..	..	..	..	.....	..
31	Winternitz: Ibid.	+	..	+	Both	..	..	..	..	.....	+
32	Winternitz: Ibid.	+	+	+	Both	..	..	..	..	Peritoneum	..
33	Wollstein and Mixsell: Arch. Pediat. **36**: 268, 1919	..	..	..	Right	..	..	..	..	.....	+
34	Gestring: J. Kansas M. Soc. **20**: 233, 1920	+	+	..	..	..	..	..	..	.....	..
35	Helvestine: J. Cancer Research **7**: 209, 1922	..	..	..	..	..	..	+	..	.....	..
36	Clawson and Cabot: J. A. M. A. **80**: 999, 1923	..	..	..	..	..	..	+	+	Pancreas	+
37	Jaffe: Arch. Int. Med. **33**: 330, 1924	..	..	..	..	..	..	..	..	.....	?
38	Friedenwald and Fried: Am. J. M. Sc. **168**: 875, 1924	..	..	..	..	..	..	..	..	.....	..
39	Fried: Am. J. M. Sc. **168**: 241, 1924	+	+	..	Both	..	..	..	..	.....	..
40	Von Glahn and Lamb: M. Clin. N. Amer. **8**: 29, 1924	+	+	+	Both	..	..	..	..	.....	..
41	Von Glahn and Lamb: Ibid.	+	..	..	..	..	..	..	..	.....	..
42	Von Glahn and Lamb: Ibid.	..	..	..	..	..	..	..	..	.....	..
43	Von Glahn and Lamb: Ibid.	+	+	+	..	..	..	..	..	.....	..
44	Rowen and Mallory: Am. J. Path. **1**: 677, 1925	..	..	..	..	..	..	..	..	.....	+
45	Counseller and McIndoe: Arch. Int. Med. **37**: 363, 1926	..	..	..	..	..	..	..	..	.....	+
46	Counseller and McIndoe: Ibid.	..	+	+	Both	..	..	..	..	.....	..
47	Counseller and McIndoe: Ibid.	..	..	..	..	..	..	..	..	.....	+
48	Counseller and McIndoe: Ibid.	..	..	..	..	..	..	..	..	.....	..

TABLE 3.—*Reported Cases of Primary Carcinoma of the Bile Duct Type from 1902 to 1926*

Number	Author and Reference	Extension into Extrahepatic Veins			Metastases						
						Lymph Nodes					
		Portal Vein	Hepatic Vein	Vena Cava	Lungs	Thoracic Duct	Mesenteric Glands	Portal Vein	Retro-peritoneum	Unusual	Bile
49	Burt: Post-Graduate **18**: 991, 1903	..	..	..	..	..	..	..	..	.....	..
50	Karsner: Arch. Int. Med. **8**: 238, 1911	..	..	..	..	..	..	+	..	Suprarenal	..
51	Karsner: Ibid.	..	..	..	Both	..	..	..	+	Pleura; kidney; pancreas	..
52	Karsner: Ibid.	..	..	..	..	..	..	..	..	.....	..
53	Karsner: Ibid.	..	..	..	Both	+	+	..	+	.....	..
54	Karsner: Ibid.	..	..	..	..	..	+	..	+	Pancreas; pleura; suprarenal	..
55	Griffith: Am. J. M. Sc. **155**: 79, 1918	..	..	..	..	..	..	..	..	Peritoneum	..
56	Dansie: Lancet **2**: 228, 1922	..	..	..	..	..	..	..	..	.....	..
57	Lichty and Richey: Ann. Clin. Med. **1**: 164, 1922	..	..	..	..	..	..	..	..	.....	..
58	Lichty and Richey: Ibid.	..	..	..	..	..	..	..	..	.....	..
59	Lichty and Richey: Ibid.	..	..	..	..	..	..	..	..	.....	..
60	Fried: Am. J. M. Sc. **168**: 241, 1924	..	..	..	Both	..	..	..	..	Kidneys	..
61	Fried: Ibid.	..	..	..	Both	+	..	..	..	Pleura	..
62	Fried: Ibid.	..	..	..	Both	..	..	..	..	.....	..
63	Fried: Ibid.	..	..	..	Right	..	..	..	..	.....	..
64	Counseller and McIndoe: Arch. Int. Med. **37**: 383, 1926	..	..	..	..	..	..	..	..	Extension to spine	+

TABLE 4.—*Unclassified Cases of Primary Carcinoma of the Liver Reported from 1902 to 1926*

Number	Author and Reference	Extension into Extrahepatic Veins			Metastases						
						Lymph Nodes					
		Portal Vein	Hepatic Vein	Vena Cava	Lungs	Thoracic Duct	Mesenteric Glands	Portal Vein	Retro-peritoneum	Unusual	Bile
65	Wells: Am. J. M. Sc. **126**: 403, 1903	+	..	..	..	..	..	..	..	.....	..
66	Ryan: Canada Lancet **38**: 1030, 1904-1905	..	..	..	..	..	..	+	..	.....	..
67	Fitzgerald: Canad. Pract. & Rev. **31**: 551, 1905	..	..	..	..	..	..	..	..	.....	..
68	Bramwell: Clin. Stud. **4**: 209, 1905-1906	..	..	..	..	..	..	..	..	.....	..
69	Ievers: J. Roy. Army Med. Corps **8**: 631, 1907	+	..	..	..	..	..	..	..	.....	..
70	Wood: Proc. New York Path. Soc. **8**: 73, 1908-1909	..	..	..	..	..	..	..	..	.....	+
71	Wheeler: Guy's Hosp. Rep. **63**: 225, 1909	..	..	..	Right	..	..	..	..	.....	+
72	Wheeler: Ibid.	..	..	..	..	..	..	..	..	.....	..
73	Wheeler: Ibid.	..	..	..	..	..	..	..	..	.....	..
74	Abbe: Ann. Surg. **54**: 236, 1911	..	..	..	..	..	..	..	..	.....	..
75	Glynn: Brit. M. J. **2**: 1192, 1911	..	..	..	Both	..	..	..	..	.....	..
76	Freeman: Lancet **2**: 157, 1914	..	..	..	..	..	..	..	..	.....	..
77	Williamston: M. Clin. N. Amer. **8**: 453, 1924	..	..	..	..	..	..	..	..	.....	..
78	Johnson: Lancet **2**: 19, 1924	..	..	..	..	..	..	..	..	.....	..
79	Schlisler and Moffit: J. Missouri M. A. **23**: 137, 1926	..	..	..	..	..	..	..	..	.....	..
80	Langley: California & West. Med. **24**: 75, 1925	..	..	..	Both	..	..	..	..	Peritoneum	..

in fourteen cases. Metastases elsewhere showed that the gallbladder was involved in eight cases. Eggel specifically stated that in these the mucosa was intact and the metastases were in the wall. The extrahepatic bile ducts and the peritoneum were each the seat of metastases in five; the omentum and kidney each in three; the heart, cranial bones and sternum each in two, and the thyroid, spleen, pylorus, diaphragm, ovary, stomach, testicle, pancreas, trachea, esophagus, pleura, suspensory ligament of the liver, colon, finger musculature, spleen, brain, sacrum and dura mater each in one case.

In our review of eighty cases, we found metastases in thirty-two (40 per cent, agreeing exactly with Eggel). The lungs were the most common site, being involved in twenty-one cases (26.3 per cent). The lymph glands were involved in twelve cases (15 per cent); the portal vein in seven; the retroperitoneum in six; the thoracic duct in three, and the mesenteric glands in two cases. As regards other metastases, four cases had metastases to the peritoneum and four to the pleura; three to the suprarenal, kidney and pancreas; two to the great omentum and extension of the primary growth to the vertebra in two cases, and one each to the spleen, eye, brain and meninges.

Ewing and Winternitz both state that extrahepatic metastases occur much earlier and are more frequent in the bile duct than in the liver cell type of carcinoma. Our tables show 16 per cent more metastases in the bile duct type.

The metastases are usually atypical and not widespread. Those that occur in the lung are usually small and not diagnosed until the post-mortem examination and often not until microscopic sections are made. The enlargement of the lymph gland usually includes only the regional lymph glands. In case 1, the new growth was in the region of the hilum, and extension of the growth into the gland was from the hilum toward the periphery. This indicates retrograde metastasis. In certain carcinomas of the liver cell type the cells retain their functional activity. Of the 163 cases compiled by Eggel, fifteen showed bile in the primary tumor and of these only three had bile in the metastases (cases of Jungmann, Heller and Schmidt). In our review of eighty cases, bile was demonstrated fifteen times in the primary growth and only four times in the metastases (cases 5, 24, 33 and 36 in table 2). Therefore, in a total of 243 cases, bile was present in the metastases of seven (2.9 per cent). Winternitz stated that the anaplasia of malignant cells is found to result in a loss of the more specific functions, and this accounts for certain liver cell neoplasms in which bile is not present. Several writers believe that the finding of bile pigment is only of relative value in differentiating the two types, as tumors arising from the small bile ducts may also have bile pigment within their lumina. However, we believe that if bile is sought in carcinomas of the liver it would

be found more frequently than is reported, and the finding of intracellular bile in the primary tumor, and certainly the finding of bile in the metastases, would justify the diagnosis of primary carcinoma of the liver cell type. To quote Eggel, "The most striking proof of the development from liver cells is the finding of bile secretion in the metastases."

Certainly the presence or absence of fat in the cells of the new growth does not give a definite idea as to the origin of the neoplasm. Glycogen, although proved to be present in some cases of carcinoma of the liver cell type, does not have important bearing on the origin, since it is a fact that other rapidly growing tumor cells frequently contain glycogen.

Conservative clinicians hesitate to make a positive diagnosis of primary carcinoma of the liver. This is due to the fact that there is not any accurate method of characterizing clinically the various lesions of the liver. Besides, this organ is frequently involved secondarily. In case 1, there was a proved primary carcinoma of the liver cell type with the patient in good condition. We hoped that the various tests involving function of the liver cells would give us useful diagnostic information. The van den Bergh, phenoltetrachlorphthalein, Graham Cole and sugar tolerance tests were made, and the bile index and quantity of amino-acid nitrogen were determined. The patient had a high (0.179) blood sugar content while fasting. There was a definite delayed excretion of the phenoltetrachlorphthalein. Other observations were within normal limits. Certainly, such information cannot be used to designate a specific type of liver injury. Green metastatic nodules, as in one case, should suggest primary carcinoma of the liver cell type. Of the eighty cases tabulated five were diagnosed by biopsy (cases 5, 23, 26, 44 and 55). The majority of such carcinomas must still be diagnosed at necropsy or from a nodule removed during a surgical exploration.

Case 1.—F. S., aged 13, entered the hospital, Nov. 12, 1926, with the complaint of distress under the right costal margin and of a lump near the midline under the right costal margin. Her illness began in November, 1925, with pain in the abdomen transmitted to the back. She became nauseated, and vomited. The entire abdomen was tender. The attack subsided in two days, but similar attacks occurred at irregular intervals afterward. The lump was noticed in the upper part of the abdomen in June, 1926. From Nov. 1, 1926, until her admission to the hospital, pain in the upper abdomen was persistent, though varying from a dull ache to a sharp pain and aggravated by coughing. It radiated toward the crest of the right ileum.

On examination the chest was found to bulge anteriorly in the region of the right lower costal margin. A slightly tender mass, which did not move with respiration, was felt under the right costal margin. Near the ensiform cartilage were two firm nodules. Roentgen-ray examination of the chest and urinary tract revealed essentially negative observations.

An exploratory operation was made on Dec. 3, 1926. Nodules were found on the dome of the liver and in the liver substance. The largest was 2.5 cm. in diameter, elevated, umbilicated, hard and grayish white. The liver extended below the level of the right iliac crest. Numerous enlarged lymph nodes were found in the upper part of the abdomen, mostly retroperitoneal. A gland was removed from the gastrohepatic omentum for diagnosis. Free fluid was not found in the abdomen.

The tissue removed appeared to be a lymph node measuring 3 by 1.5 by 1.5 cm. It was grayish pink and of a uniform soft consistency except for three pea-

Fig. 1.—Photomicrograph representing histology of bile duct type of carcinoma; × 500.

green nodules which were hard. On cut section the nodules were sharply demarcated but firmly attached to the adjacent tissue. The nodules varied from 3 to 6 mm. in diameter. They were composed of a cellular somewhat friable tissue. The cut margins remained distinctly angular.

Numerous sections were made from various parts of the specimen. Phosphotungstic acid, Mallory's aniline blue, silver impregnation and hemotoxylin and eosin methods were used. The section consisted for the most part of lymphoid tissue. The nodules were made up almost entirely of epithelial tumor cells which histologically closely resembled liver cells. Similar cells were found in small areas scattered throughout the lymphoid tissue. Many lymphatics were

filled with neoplastic cells. The infiltration extended inward from the hilum of the lymph node. Tumor tissue was not present in the region of the peripheral sinus. The tumor cells varied in size from three to six times the diameter of a normal, adult red blood cell. The neoplastic cells were roughly polygonal in shape. They were sharply defined, with a finely granular cytoplasm which stained quite deeply. In the nuclei of many of the cells were rounded areas surrounded by a somewhat vesicular zone, these undoubtedly were degenerative nuclear inclusions. The nuclei were round and prominent. Many were swollen and vacuolated. Mitotic figures were not found. These atypical liver cells

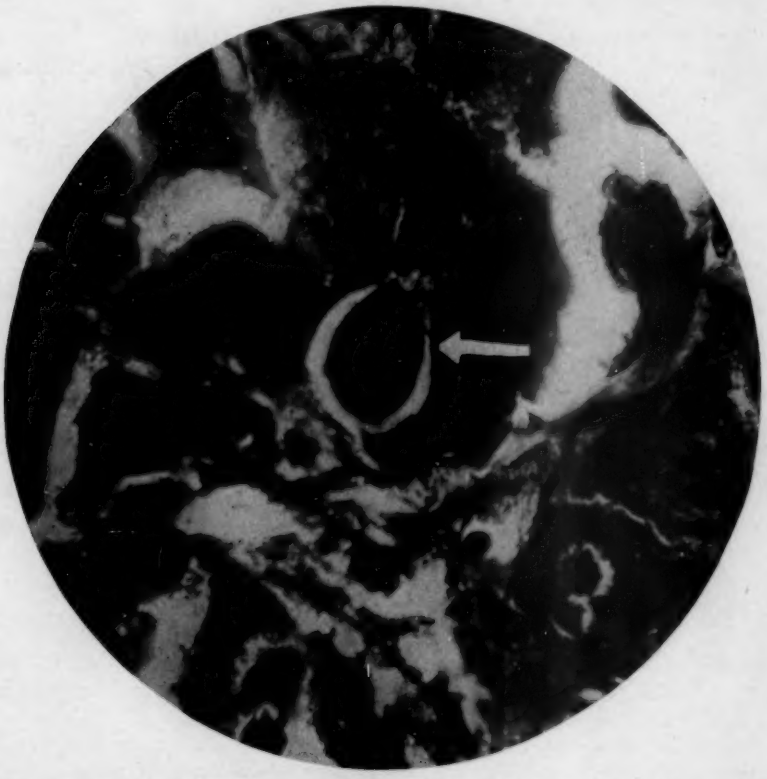

Fig. 2.—Photomicrograph from metastatic nodule in lymph node; × 1,000. Marked bile stasis, both intracellular and extracellular is present. The largest mass of inspissated bile is indicated by the arrow.

formed a compact parenchymatous structure. Frequently the cells were seen arranging themselves into cords, and occasionally the cords assumed a spoke-like figure. The spokes radiated outward from a central opening similar to the central portion of a liver lobule. No vessel walls could be seen surrounding the central opening, or between the cords of liver cells. In various sections of the tumor, bile was seen within the cytoplasm of the tumor cells, and occasionally between the cells themselves. Bile capillaries were not present. Mallory's aniline blue, phosphotungstic acid and hemotoxylin stains showed a scanty amount of connective tissue stroma.

April, 1927 (three months after operation), sugar tolerance tests (35 Gm. of dextrose) gave the following results:

Hours	0	½	1	1½	2.0	2½
Blood sugar....	179	179	234	213	190	140

The urine was free from sugar. The van den Bergh reaction was negative. The bile index was normal.

In the phenoltetrachlorphthalein test of liver function the following results were obtained: 8 per cent of the dye remained in the blood after fifteen minutes; 6 per cent of the dye remained in the blood after one hour; 4 per cent of the dye remained in the blood after two hours.

The blood chemistry was as follows: creatinine, 0.85; uric acid, 2.5; amino acid nitrogen, 8.7.

The basal metabolic rate was 13.2.

Diagnosis: Primary carcinoma of the liver, liver cell type, with bile containing metastases in the abdominal lymph glands.

Case 2.—E. C. A., aged 55, entered the hospital, May 14, 1926, complaining of weakness, pain in the abdomen, vomiting and a queer feeling in the head. His trouble began about two or three years previously with pain in the region of his stomach. Since that time his appetite had been poor and he had had occasional attacks of vomiting. The patient was delirious on admission.

Fulness of the abdomen, most marked in the epigastrium, and a palpable nodular mass in the epigastrium which extended downward to the level of the umbilicus and to the right almost to the crest of the ileum, were noted. On May 19 an exploratory operation revealed an enlarged, dark red, soft, boggy liver, with numerous scattered grayish areas beneath the surface. An irregular firm mass was felt between the liver and the stomach. The patient died two days after the operation.

At necropsy the peritoneal cavity contained 500 cc. of bloody fluid. The liver weighed 2,790 Gm. The outer surface presented numerous elevated nodules varying from a few millimeters to 3 cm. in diameter. On cut section the nodules were found to be composed of a gray, soft and friable tissue. One nodule measured 15 cm. in diameter, and its central portion showed an area of necrosis. The involvement was most marked in the left lobe. Bleeding into the peritoneal cavity had taken place from the spontaneous rupture of a nodule on the anterior surface of the right lobe. The gallbladder was small and thickened, and contained a gallstone measuring 1 cm. in diameter. The cystic duct was occluded by a stone. The remainder of the organs were examined and no evidence of tumor was found in them. The sections of liver all contained masses of tumor cells. The liver cells surrounding the tumor masses showed evidence of atrophy due to pressure. The neoplastic cells closely resembled liver cells, being large (from four to seven times the diameter of a normal red blood cell), granular and polygonal. The nuclei were large and vesicular. Mitotic figures were not found. The tumor cells formed a parenchymatous structure with a slight tendency toward the formation of a cord in the liver. Bile was not found and bile capillaries could not be demonstrated. The connective tissue was scanty. The lymphoid tissue around a bronchiole within the parenchyma of the lung was infiltrated with tumor cells similar to those found in the liver. The lymphatics were found to be occluded with neoplastic cells. The remainder of the lung was normal except for some congestion of the capillaries.

Diagnosis: Primary carcinoma of the liver, liver cell type, with metastasis to the lung (microscopic).

CASE 3.—W. F., aged 68, an unmarried man, entered the hospital, May 25, 1915, complaining of stomach trouble and jaundice. He had had typhoid in September, 1913, and was in bed for seven weeks. Following this he began having a feeling of heaviness in his stomach after meals. In May, 1914, pain accompanied this heaviness. In March, 1915, a lump was noticed in the epigastrium which rapidly enlarged for one month. The patient lost 51 pounds (23 Kg.) during his two years of illness.

The patient was deeply jaundiced. The upper part of the abdomen bulged forward in the region of the ensiform cartilage and right costal margin and 13 cm. below the ensiform cartilage. It was slightly tender. The edge of the spleen extended 3.5 cm. below the costal margin. The stool was of a putty-like consistency and color. Blood was not present. Bile was present in the urine (4 plus chemical test).

On May 28, 1925, two small firm subcutaneous nodules developed over the anterior aspect of the right upper part of the chest. These nodules were freely movable. On June 7, 1915, one of the nodules was removed, and a diagnosis of carcinoma was made. On June 15, 1915, two more nodules appeared. A sharp pain developed under the right costal margin which was transmitted to the left shoulder and neck. On June 25, 1915, the patient died.

At necropsy the skin, conjunctivae and mucous membranes were bright yellow. The dorsum of the left foot was swollen. A number of subcutaneous nodules were palpated. These were distributed over the right scapula and sternum, under the right costal margin and over McBurney's point and the os pubis. The peritoneal cavity contained a moderate amount of dark reddish-brown fluid. A few dense adhesions were found between the liver and the diaphragm and between the liver and the hepatic flexure of the colon. Numerous enlarged lymph nodes were present along the small intestine and in the mesentery, none of which exceeded a diameter of 2 cm. The outer surface of the liver was greenish and was studded with elevated umbilicated nodules. The consistency of the entire liver was more firm than normal. On cut section little normal liver tissue was seen. The nodules in the tumor were most numerous in the right lobe. Numerous nodules were distributed throughout the organ, the largest nodule measuring 1.5 cm. in diameter. The gallbladder was filled with gallstones. The extrinsic bile ducts did not contain tumor tissue. Enlarged lymph nodes were found around the aorta in the region of the renal veins, also at the hilum of both lungs. The upper portion of the pericardial sac and the outer surface of the heart presented several flattened nodules similar to those found in the liver. The largest of these nodules measured 0.5 cm. in diameter. The left ventricle measured 2.5 cm. in thickness. In the wall of the left ventricle were several small whitish nodules. The valves were normal except for a small nodule on the posterior cusp of the mitral valve measuring 1 mm. in diameter and resembling the nodules found in the myocardium and the liver. The spleen was enlarged and soft, and on cut section deep red. The sections of the liver presented little normal liver tissue. A few fragmented islands of parenchymal cells were densely infiltrated, for the most part by yellowish-brown granules. Scattered throughout the sections were nodules of neoplastic cells. These cells were cuboid and arranged themselves into nests and rows and occasionally into ducts. Definite lumina were formed. The cells varied somewhat in size, many of them being larger than normal bile duct epithelium, but the average was about the size of normal intrinsic bile duct

epithelium. At the center of the nodule the cells were necrotic. The pancreas presented an increase of fibrous tissue. The small amount of tumor tissue was separated from the pancreatic tissue by a fibrous wall. The heart muscle contained nodules of tumor cells corresponding in all respects to those found in the liver and pancreas. The cross striations of the heart muscle cells were indistinct, and the cytoplasm at either pole of the cell showed a deposit of yellowish-brown granules. The nuclei were swollen and vacuolated. The origin of this tumor was undoubtedly the epithelium of the intrinsic bile ducts as evidenced by the type and arrangement of the cells and the amount of stroma present.

Diagnosis: Primary carcinoma of the liver, bile duct type, with multiple metastases.

SUMMARY

The incidence of primary carcinoma of the liver is 0.13 per cent at the hospital of the State University of Iowa. This incidence is based on 1,500 necropsies.

The morphology, arrangement and function of cells are the important factors in the histologic diagnosis of primary carcinoma of the liver.

Vascular invasion is a prominent feature of primary carcinoma of the liver.

The various tests that involve liver function do not have any specificity in the clinical diagnosis of primary carcinoma of the liver.

MIXED TUMORS OF THE KIDNEY

REPORT OF FOUR CASES *

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It seems worth while to call attention to mixed tumors of the kidney, not on account of their comparative rarity, but because they are seldom considered clinically, although the interesting pathologic features have many clinical bearings.

The credit is generally given to Birch-Hirschfeld [1] for recognizing that tumors of this variety have characteristics in common, notwithstanding their variance in histologic structure. However, nearly ten years before, Jacobi [2] concluded that they are a distinct form of tumor.

As a rule, they are noted during fetal life or in the first few years of postnatal existence. Walker [3] said that they are congenital or occur most frequently during the first and second years of life. In reviewing all available reports, he noted that 48 per cent occurred within the first two years of life, the number diminishing as the age of the patient increased, so that the incidence is 8 per cent at 5 years of age; 1 per cent at 8 years and 0.6 per cent between the ages of from 10 to 12 years.

Sex seems to be of comparatively little importance; 42 per cent of these tumors occurred in males; 39 per cent in females, and the sex of 18 per cent of the patients was not stated. Heredity could be considered as an etiologic factor in only 5 per cent of the patients.

Trauma, irritation and acute infectious diseases have an etiologic importance in that 22 per cent tumors developed in patients who gave a history of preceding trauma. There is one report of a mixed tumor of the kidney which developed one year after the passage of renal calculi, and in five patients the occurrence of the tumor was preceded by one of

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1. Birch-Hirschfeld, F. V., and Döderlain, A.: Embryonale Drüsengeschwulst der Nierengegend im Kindesalter, Centralbl. f. d. Krankh. d. Harn- u. Sex.-Org. **5**:3, 1894. Birch-Hirschfeld, F. V.: Sarkomatöse Drüsengeschwulst der Niere im Kindesalter (Embryonales Adenosarkom), Beitr. z. path. Anat. u. z. allg. Pathol. **24**:343, 1898.

2. Jacobi, A.: Primäres Sarcom der Niere bei dem Foetus und dem neugeborenen Kinde, Jahrb. f. Kinderh. **25**:112, 1886. (Read before the Eighth International Congress for Medical Sciences at Copenhagen, 1884.)

3. Walker, G.: Sarcoma of the Kidney in Children: A Critical Review of the Pathology, Symptomatology, Prognosis, and Operative Treatment as Seen in One Hundred and Forty-Five Cases, Ann. Surg. **24**:529, 1897.

the exanthematous fevers. Weigert [4] said that the tumor tissue may be stimulated to proliferate by disease, which also lowers the inhibitory influence of the kidney.

Birch-Hirschfeld [1] said that these tumors arise from remnants of the wolffian body. Wilms [5] disputed this, because the wolffian body does not contain any striated muscle fibers which are so often present in the tumors. He maintained that these tumors arise from an inclusion of undifferentiated mesodermal tissue which later gives rise, in the course of normal development, to the pronephros and wolffian body and to the myotomes. This undifferentiated embryonal tissue, when it proliferates, may produce the tubules that simulate those in the wolffian body; it may produce the embryonal muscle cells so often present in these growths, and likewise, if a portion of the sclerotome is carried along, cartilage also may be found. These tumors thus contain a mixture of tissues, any of which may predominate.

These tumors always develop inside the kidney; the kidney tissue takes no part in the tumor formation, but undergoes pressure atrophy. In one report, the tumor is described as cystic with several pints of fluid in the cysts.

These mixed tumors are peculiar in forming few and insignificant metastases in comparison with the large size of the primary tumor. They develop almost exclusively during the fetal period or in the first few years after birth, grow rapidly and produce marked cachexia.

Microscopic examination shows that the majority of these tumors resemble sarcomas. Many contain both round and spindle cells, some have an adenomatous structure, others contain a few muscle fibers, and a lesser number contain cartilage.

REPORT OF CASES

CASE 1.—The body of a child, aged 1½ years, was examined to ascertain the cause of death (coroner's examination), and a tumor was found in the right kidney. The lower pole of the kidney was continuous with the tumor mass which was the shape of a slightly flattened sphere, and measured 12 cm., in its greater diameter and 7 cm. in the lesser. The entire mass was covered with a capsule underneath which were prominent distinct blood vessels. Numerous tubules were found microscopically (fig. 1).

CASE 2.—A boy, aged 10 months, was well until three weeks before admission to the hospital with a condition diagnosed as gastro-enteritis. A tumor was found and removed, and the patient died one month later. The tumor was elliptical and about 14 cm. long, and a microscopic examination showed that the tubules were abundant.

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4. Weigert, Carl: Adenocarcinoma renum congenitum, Virchows Arch. f. path. Anat. **67**:492, 1876.

5. Wilms, M.: Die Mischgeschwülste, Leipzig, Arthur Georgi, 1899.

CASE 3.—A tumor was removed from a man, aged 45 years, who had suffered with frequency of urination and polyuria for three years. Eight months before admission to the hospital, he noticed pain in the right lumbar region, which radiated downward to the hip and thigh, and upward to the middle of the chest. Two months later, he developed a right-sided varicocele. One month later, the patient began to have intermittent hematuria, and on examination a marked enlargement of the right kidney was found. At the time of operation, metastases in the lymph glands along the aorta and vena cava were noted. The patient died one year after the operation.

The tumor mass weighed 400 Gm. and had a soft pulplike consistency. The surface was nodular. Microscopically, there were tubules and embryonal striated muscle cells (fig. 2).

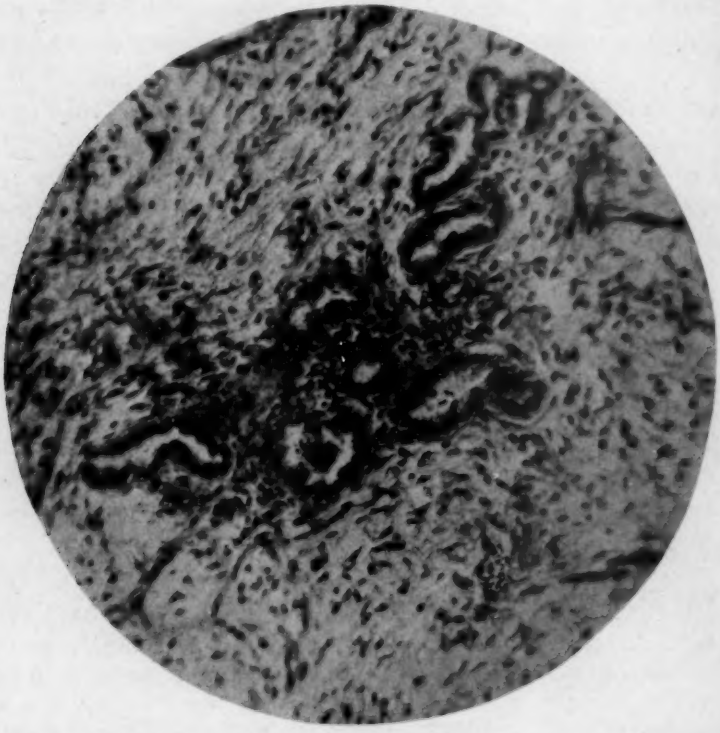

Fig. 1.—The tubules which have been responsible for the name "adenosarcoma" sometimes given these tumors are said to have their origin in the wolffian body.

CASE 4.—*History.*—A school boy, aged 9, entered the hospital with the following history. When 7 months old, he fell from a hammock, striking his left side on the edge of a bed. Since the accident he had always been sick. When about 4 years old, he began to have severe colicky pains in the left side, which would last from a few hours to a day and even longer. At this time, the patient lost from 25 to 30 pounds (11.3 to 13.6 Kg.) in weight. Five months before admission to the hospital a diagnosis was made of tumor of the abdomen which probably involved the left kidney. The tumor had increased in size. A month before admission, the patient had epileptiform convulsions which lasted an hour and

which were present during his stay in the hospital. He had a headache continuously. An exploratory laparotomy was performed, but due to the extensive adhesions about the tumor mass on the left side, the growth was not removed. The patient died two months later.

Necropsy.—Necropsy was performed eight hours after death, and the anatomic diagnosis was as follows: "Mixed tumor (Wilms) of the left kidney; regional involvement of the left adrenal, inferior vena cava, pancreas, transverse duodenum and descending colon; metastatic tumor growths in both surfaces of the diaphragm; subpleural tumor growths opposite the lower ribs; stenosis of the right ureter; dilatation of the left ureter and renal pelvis; anemia of the right kidney; enlargement of the tracheobronchial and perioesophageal lymph glands; hypostatic congestion in both lower lobes (slight); ascites; general anemia."

Fig. 2.—The embryonic striated muscle fibers, phosphotungstic acid hematoxylin stained.

The following description was made: "In the tumor mass as removed from the body, are included the pancreas, left kidney and adrenal, large intestine, aorta and vena cava. It is considerably larger than a man's head and resembles a cube with its edges and corners irregularly knocked off. Its contour is too irregular to call it a sphere, and its diameters too nearly equal to call it elliptical, oval or pyriform. On its anterior surface it is rounded where it projects forward into the abdominal cavity, and it is hollowed out posteriorly where the vertebral column fits into it. The abdominal aorta, which is completely surrounded by the tumor mass, serves as a convenient landmark for measurements. To the left of it the tumor extends 11.5 cm., to the right 9.5 cm., posteriorly from 3 to 3.5 cm., and anteriorly from 6 to 8 cm. The aorta, though completely embedded, is thus seen to lie much nearer the posterior than the anterior margin of the growth. The length from above downward is 20 cm. The tumor consists mainly of a mass of

nodules varying from the size of a pinhead to that of two fists, a mass of the latter size lying in front of the left kidney and forming the anterior lower left corner of the growth. The left kidney is 14 by 8 by from 4 to 5 cm.

"The mesentery of the small bowel is confluent with and indistinguishable from the tumor tissue, except for a short distance on the right anterior surface of the mass. The free border of this portion is 27 cm. in length and its attachment 7 cm. It is thickly studded with tumor nodules. These tumor nodules are mainly firm, but several regions of softening and liquefaction are on the external surface and on the cut section. The firm tumor tissue is whitish; the soft places are pink or red. At several places in the tumor there is blood pigmentation, showing shades from light to dark green and of purple. The posterior surface of the mass shows considerably more of a tendency than does the anterior surface.

"The colon is embedded in the mass, and is considerably constricted, especially in its transverse and descending portion. The tumor tissue has penetrated its wall and in places almost hangs free in the lumen. The sigmoid is also involved by the lower portion of the mass. The pancreas is embedded in the upper posterior portion of the growth and is difficult to isolate. The vena cava is surrounded on all sides by tumor tissue from which it cannot be separated, being completely fused with it. The lining appears at first sight entirely smooth and unaffected, but closer inspection reveals a small area on its anterior wall near the middle of its course where a small slender soft, bloody growth 2 cm. long projects into the lumen. Apparently it does not come from one of the renal veins, but results from a direct piercing of the wall by the new growth. The wall in the vicinity is considerably reddened. Further inspection reveals four or five other similar places where the tumor nodules in the wall produce more or less marked bulging of the intima but have not yet broken through into the lumen."

On surfaces made by cutting, "there is a tract of compact tissue of a fibrillar appearance extending the whole length of the specimen. There are branchings extending out from this which cut off various shaped islands of tissue, some of which are extremely soft while others are of a firm consistency. Some of these spaces contain large firm yellowish white retroperitoneal glands. In the lower and anterior portion of the specimen is a portion of the tumor comprising about one-third of its bulk which seems not to be invaded by these strands. Along its inner border it is white and of a mushy consistence and passing toward the periphery, it becomes firmer and darker, finally attaining a slate color. Several blood vessels measuring from 2 to 4 cm. in diameter appear on the surfaces made by cutting. In one instance these strands of tissue enclose the vessel wall sending in processes which blend with the adventitia of the vessel."

"The histologic examination of the sections of the liver, prostate, seminal vesicles and thymus reveal no alterations. There are marked alterations in the sections from the lungs. The vessels generally contain micrococci and the alveoli contain a fluid that has been coagulated by the fixing agents. In the spleen the sinuses are generally empty, and cocci occur in many places and the tissue about them is unchanged. There is an irregular thickening of the tunica media in the small arterioles, the added tissue being homogeneous and possesses a marked affinity for eosin. In the pancreas, tumor cells may be found within the small veins and venules in sections that contain no tumor cells elsewhere. The myocardium is made up of slender muscle fibers and the perivascular loose connective tissue has the appearance of being separated by

an edema, for a finely granular, non-staining material fills all the interfibrillar spaces.

"The tumor tissue in the lymph glands, pancreas, diaphragmatic and pericostal nodules and from various places in the primary growth has a similar structure. As seen with the lower power, the cells are little more than masses of chromatin. On closer examination the chief feature of the cells is their polymorphism. The average tumor cells nucleus is larger than a red blood corpuscle. There are also numerous giant nuclei and less frequently aggregated nuclei that bear slight resemblance to giant cells. All the tumor cells possess but little cytoplasm. Many tumor cells, especially in sections from some parts of the primary growth, possess fragmented nuclei. Nuclei in various stages of mitosis are also frequent. Such tumor cells are grouped in rather large clusters separated by bands of fibrous tissue and this arrangement is best shown with a hand lens of a few diameters amplification. The fibrous bands contain cells with spindle-shaped nuclei sparsely distributed. Capillaries are abundant in the tumor tissue, and vessels with heavier walls in the stroma. Cocci are frequently found in the capillaries and the occasional poor staining of the adjacent cells and tissue give the impression of an antemortem invasion."

SUMMARY

1. These tumors usually occur during the first two years of life; they are encapsulated and give rise to few metastatic growths, and only when the primary tumor is large.

2. The first tumor always develops within the kidney, and the kidney does not take any part in the tumor formation, but undergoes a pressure atrophy. Hematuria occurs late in the disease.

3. One growth here reported was associated with trauma, another was encountered in a man 45 years old, and in one patient the hematuria was first noticed nearly three years after the first symptoms caused by the tumor.

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LESIONS IN THE PULMONARY ARTERY AND VALVE ASSOCIATED WITH RHEUMATIC CARDIAC DISEASE *

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At present, the Aschoff nodule, with its characteristic accumulation, distribution and structure of cells, is the only generally conceded specific lesion in rheumatic heart disease. These nodules have been found not only in the ventricular myocardium, but also in the auricles and heart valves and in the inflamed pericardium.[1] Recently, Pappenheimer and von Glahn [2] directed especial attention toward the blood vessels in rheumatic fever. Previous observers reporting involvement of the aorta failed to furnish satisfactory histologic proof of the rheumatic infection, the only important exception being Klotz in his paper published in 1912.[3] In their first communication on the subject of rheumatic aortitis, Pappenheimer and von Glahn [4] described Aschoff bodies or Aschoff cells in the adventitia of the aorta and healed lesions consisting of compact, cell-free, flame-shaped scars in the media. In their second publication,[5] they described changes representing the acute stage of the medial lesions previously reported. In the outer two thirds of the media they noted conspicuous collections of lymphocytes, polymorphonuclear leukocytes and Aschoff cells about the nutrient arterioles which were themselves greatly altered. In their most recent paper,[6] they described, in addition, intimal and subintimal cellular infiltrations in the aorta which in places were extensive enough to produce distinctive plaques on the inner surface of the vessel. These authors suggested that the intimal lesions probably represented invasion of the artery from the lumen, whereas the medial and adventitial lesions could be explained by assuming the entrance of infection by way of the vasa vasorum.

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4. Pappenheimer and von Glahn (footnote 2, first reference).

5. Pappenheimer and von Glahn (footnote 2, second reference).

6. Pappenheimer and von Glahn (footnote 2, fourth reference).

A search of the literature revealed only a few reports on the involvement of the pulmonary artery in the course of rheumatic cardiac disease. Gotthardt [7] reported a case of verrucose endocarditis of all the valves and fibrinous pericarditis which was accompanied by endarteritis of the pulmonary artery. Bacterial stains of the vegetations on the pulmonic valve were negative for tubercle bacilli and other organisms. This report was made before the specific lesions of rheumatic fever were described, but the description of the case indicates that it was probably one of acute rheumatic infection.

Wätjen,[8] in a case of rheumatic heart disease and extensive pericarditis, noted the presence of Aschoff bodies in the epicardial fat over the anterior surface of the pulmonary artery. Sacks,[1] in his review of the pathology of rheumatic fever, commenting on the finding of Aschoff bodies in the wall of the aorta, stated that one would also expect to find the specific lesions in the pulmonary artery, because of the common origin of their nutrient vessels from the coronary arteries. Later, Paul [9] reported a case of acute rheumatic fever in which histologic examination of the pulmonary artery revealed an extensive destructive lesion in the media, with hyalinization and scarring and Aschoff bodies in the adventitia.

MATERIAL AND METHODS

The present study was undertaken to determine the frequency and type of inflammatory changes in the pulmonary artery [10] and valve and in the adjacent fibrous ring in cases of rheumatic heart disease.

In the selection of cases for the rheumatic group, we have used the following criteria:

1. A clinical history of one or more attacks of rheumatic fever or chorea, or of repeated attacks of tonsillitis, migrating arthritis, or the presence of rheumatic subcutaneous nodules.
2. The observation of a verrucose endocarditis, showing the characteristic structure, with absence of bacteria in sections.
3. The observation of Aschoff bodies in the myocardium.
4. The presence of a universal fibrinous pericarditis.
5. Mitral stenosis, nonatherosclerotic in origin.

The rheumatic cardiac cases have been further subdivided into two groups: (1) cases in which there was clinical evidence of active

7. Gotthardt, J.: Inaug. Diss., München, 1896, cited by Posselt; Lubarsch and Ostertag: Allg. Path. u. path. Anat. des Mensch. u. Tiere. **13**:407, 1909.

8. Wätjen: Verhandl. d. deutsch. path. Gesellsch. **18**:223, 1921.

9. Paul, J R: Proc. Path. Soc.; abstr. Arch. Path. **3**:354 (Feb.) 1927.

10. Changes in the smaller branches of the pulmonary artery were reported by von Glahn and Pappenheimer (footnote 2, third reference). These will not be considered in this paper.

rheumatic infection and the presence at autopsy of acute verrucose endocarditis or fibrinous pericarditis of the typical rheumatic variety, and (2) cases of chronic rheumatic cardiovalvular disease in which there were not any clinical or pathologic signs of active rheumatic infection.

In all the cases except one in the rheumatic group there was a negative history of syphilis and a negative Wassermann reaction. Cases of rheumatic cardiac disease complicated by acute or subacute bacterial endocarditis were not included, for it is obvious that lesions in the pulmonary artery could arise from either the antecedent rheumatic or the superimposed bacterial infection.

As controls, we use two groups of cases in which there were no criteria of rheumatic cardiac disease. The first group was a series of cases of pericarditis of bacterial, including tuberculous, origin or due to uremia. These cases were studied to ascertain the possibility of involvement of the pulmonary artery by direct extension from the pericardium. The second series included a group of routine cases in which neither pericarditis nor rheumatic cardiovalvular disease was present.

The sections of the pulmonary artery were taken to include the artery, valve and myocardium at the root of the artery and at various sites as far as the insertion of the vessel into the lung. The sections were stained with (1) hematoxylin and eosin, (2) Weigert's elastic tissue stain, followed by hematoxylin and Van Gieson's picrofuchsin, (3) Unna-Pappenheim's methyl-green-pyronin, (4) MacCallum-Goodpasture's bacterial stain, (5) Weigert's fibrin stain and, in some instances, (6) Giemsa stain.

CHANGES IN RHEUMATIC CARDIAC DISEASE

The accompanying table is a detailed study of the cases in which there was clinical and pathologic evidence of active rheumatic infection. In these twenty-four cases, Aschoff bodies were present in the myocardium in twenty instances, an incidence of 83.3 per cent.

A. Musculo-Arterial Junction.—The most constant lesions were found at the root of the pulmonary artery, where the artery and valve are inserted into the fibrous ring. At this point the pulmonary artery sends out strands of connective tissue between the muscle fibers; we have designated this area the musculo-arterial junction (fig. 1).

Involvement of the musculo-arterial junction was noted in seventeen of twenty-four instances, or in 70.9 per cent of the cases in which there was active rheumatic infection. In this area, we noted two types of reaction—the one consisting of Aschoff nodules, and the other of diffuse cellular infiltrations composed of lymphocytes, polymorphonuclear leukocytes, large mononuclear wandering cells and multinuclear cells

Detailed Study of Cases with Clinical and Pathologic Evidence of Active Rheumatic Infection

Autopsy Number	Age	Sex	Clinical Data: Sore Throat or Tonsillitis	Clinical Data: Migrating Arthritis	Clinical Data: Subcutaneous Nodules	Clinical Data: Past History of Rheumatic Fever	Lesions in Myocardium: Aschoff Bodies	Lesions in Endocardium: Acute Verrucose Endocarditis; Valves Involved	Lesions in Endocardium: Chronic Valvular Disease*	Lesions in Endocardium: Auricular Lesions	Lesions in Pericardium: Acute Fibrinous Pericarditis	Lesions in Pericardium: Chronic or Adhesive Pericarditis	Lesions in the Pulmonary Artery: Musculo-Arterial Junction	Lesions in the Pulmonary Artery: Pulmonary Valve	Lesions in the Pulmonary Artery: Wall of the Artery	Lesions in the Pulmonary Artery: Pericardium Over Anterior Surface of Pulmonary Artery	Lesions in the Aorta: Fibrous Ring*	Lesions in the Aorta: Aortic Valve*	Lesions in the Aorta: Wall of Artery	Cause of Death*
X-357	13	M	:	+	:	+	+	MAT	—	+	+	+	+	+	+	+	+	+	+	C. F.; rheumatic fever
5288	38	F	+	+	:	—	+	T	MAT	+	+	—	—	—	—	—	S	—	—	Cardiac failure
5340	33	F	+	+	:	:	—	T	MT	+	+	+	—	—	—	—	—	—	—	Cardiac failure
5341	14	F	:	+	:	:	+	MATP	MA	+	+	—	+	+	—	+	+	+	—	Cardiac failure
5407	17	M	:	+	:	:	+	MAT	MA	—	+	—	—	—	—	—	+	+	—	Cardiac failure
5447	12	F	:	+	:	+	+	MAT	MA	+	+	+	+	+	S	+	+	+	—	C. F.; bronchopneumonia
5456†	12	F	+	+	+	:	+	MATP	M	+	+	+	+	+	—	+	+	+	—	C. F.; rheumatic fever
5533	45	M	—	—	:	+	—	MT	M	—	—	—	—	—	—	—	—	—	—	C. F.; bronchopneumonia
5508	6	F	:	:	:	:	+	MAT	MA	—	—	—	+	+	—	—	+	+	—	Cardiac failure
5573	7	M	+	—	+	—	+	MAT	MAT	+	+	—	+	+	—	+	+	+	—	C. F.; rheumatic fever
5698	19	M	—	+	:	:	+	M	—	—	+	—	+	+	+	+	+	+	+	C. F.; rheumatic fever
5707	36	M	:	:	:	:	—	T	MAT	—	—	—	—	—	—	—	S	S	—	Cardiac failure
5807	58	F	:	:	:	:	+	MATP	MA	+	—	—	+	+	—	—	+	+	—	Cardiac failure
5810	19	F	:	+	:	:	+	MA	—	+	—	+	—	—	—	—	+	+	—	Tuberculosis, lungs, intestines
5811	14	F	+	+	:	+	+	MAT	MA	+	+	—	+	+	+	+	+	+	—	C. F.; rheumatic fever
5812	20	M	—	:	:	+	+	M	MA	—	—	—	+	+	—	—	S	S	—	Cardiac failure
5814	15	M	—	+	:	+	+	MAT	MA	+	+	—	+	+	—	+	+	+	+	C. F.; rheumatic fever
5822	25	F	+	+	:	+	+	MATP	MATP	+	+	—	+	+	+	+	+	+	+	C. F.; rheumatic fever
5827	8	F	+	+	+	+	+	MATP	MA	+	+	+	+	—	—	+	+	+	—	C. F.; rheumatic fever
5855	32	F	—	+	:	+	+	T	MAT	+	+	—	—	—	—	—	S	S	—	C. F.; rheumatic fever
5890	37	M	+	+	:	:	—	MATP	MAT	—	—	+	+	+	—	+	+	+	—	Cardiac failure
5990‡	29	M	:	+	:	+	+	MAT	MA	—	—	—	+	—	—	—	+	+	—	C. F.; bronchopneumonia
6075	13	F	+	+	—	—	+	MA	—	+	+	—	+	+	+	+	+	+	+	C. F.; rheumatic fever
6082	10	M	+	+	:	—	+	MA	—	+	+	—	+	—	—	+	+	+	—	C. F.; rheumatic fever

* M, mitral valve; A, aortic valve; T, tricuspid valve; P, pulmonic valve; S, scarring or fibrosis; C.F., cardiac failure

† This patient had a positive Wassermann reaction; the mother gave a history suggestive of syphilis.

‡ History of chorea.

of the kind seen in the Aschoff bodies. This diffuse type of reaction was the one more regularly observed. It was often conspicuous, extending from the adjacent myocardium into the fibrous ring, and into the base of the artery and valve. The diffuse cellular collections had a tendency to be more concentrated about the blood vessels but otherwise they did not have any characteristic architecture. At times, Aschoff nodules were found in the midst of the diffuse cellular infiltrations (fig. 2).

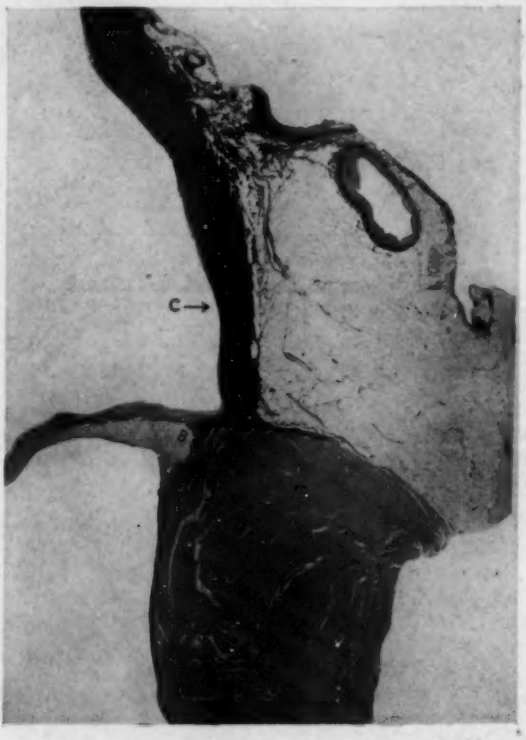

Fig. 1.—Section through pulmonary artery, valve and musculo-arterial junction to show the normal histologic appearance of this area. *A* indicates musculo-arterial junction; *B*, root of the pulmonary valve; *C*, pulmonary artery. (Figures 3 to 6—the intima is to the left.)

B. Pulmonary Artery.—In two of the twenty-four cases of active rheumatic cardiac disease there were gross changes consisting of small, raised, dull grayish-yellow patches of irregular outline which resembled the lesions found in the aorta by Pappenheimer and von Glahn. Histologic examination of the artery in both of these cases and in three additional ones, revealed active inflammatory changes. In all five the intima was thickened owing chiefly to the accumulation of small and

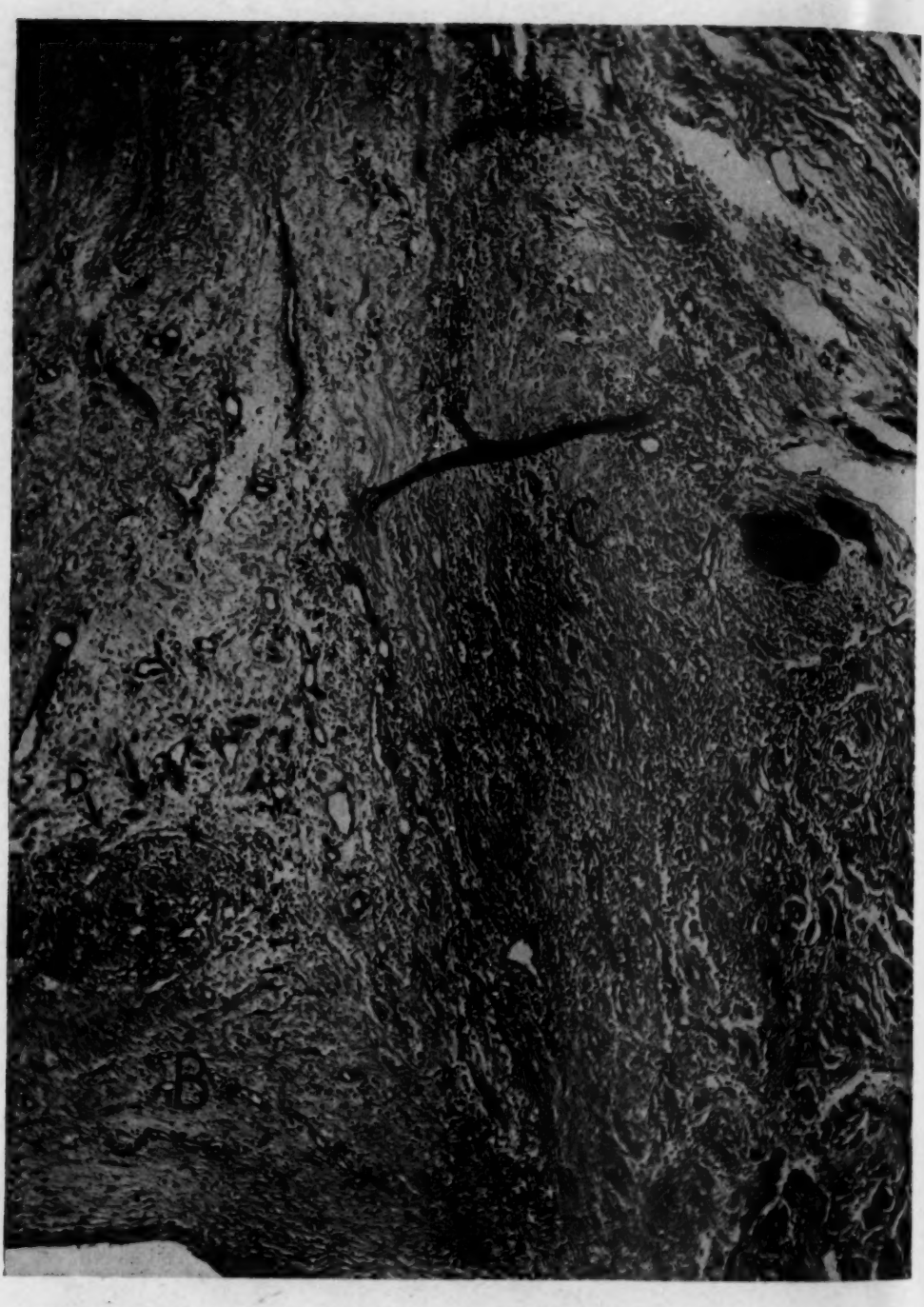

Fig. 2 (autopsy 5,447).—Extensive diffuse cellular infiltration with lymphocytes, large mononuclear wandering cells, polymorphonuclear leukocytes and Aschoff cells. Aschoff body (*D*) in the root of the pulmonary valve. *A*, musculo-arterial junction; *B*, root of pulmonary valve; *C*, pulmonary artery (root).

large mononuclear cells. In one of these cases there was necrosis of the fibro-elastic tissue of the intima, and both the intima and subintimal layers of the media were invaded by large numbers of inflammatory cells, many of which were necrobiotic, their nuclei presenting a pyknotic, distorted appearance (figs. 3 and 4). This picture closely approximated the lesion found in the auricle by von Glahn. In the media, the cells were often arranged in parallel rows or layers as though their position was limited by the underlying elastic lamellae.

The most significant lesions in the pulmonary artery were those in the media which were analogous to the observations of Pappenheimer and von Glahn and Klotz in the aorta. The entire medial wall was involved in all five cases, the more frequent type of lesion having been a cellular infiltration about the vasa vasorum. In three instances the foci in the media resembled the specific nodules found in the myocardium (fig. 5). In some of the cases there was a diffuse cellular reaction, apparently without relation to the nutrient vessels, consisting of lymphocytes, plasma cells, large mononuclear cells and multinuclear cells with vesicular nuclei. In one case, groups of large and small mononuclear cells and polymorphonuclear leukocytes radiated downward from the intima in double rows into the deeper layers of the media (fig. 3).

The alterations in the elastica varied from small focal areas of perivascular scarring to destructive changes as severe as those seen in syphilis. In the two cases in which more extensive changes were observed, the disruption of the elastica was focal and irregular in distribution, the more conspicuous changes usually surrounding a perivascular scar (fig. 6). In addition, the musculature in these cases showed nuclear depletion and, in many areas, fibrous replacement (fig. 3. Scarring of the media so characteristic of syphilis can thus be almost as intense in rheumatic fever, but puckering of the vessel was not observed.

The medial vessels, which at times extended into the inner third of the media, were often thickened, the elastica showing increased lamellation, and the intima, proliferation to the point of partial occlusion of the lumen. In some places there was appreciable scarring of the media with marked disruption of the elastica, but without much cellular reaction. It is possible that this scarring may have been the end-result of the active inflammatory process described previously. The same artery sometimes showed both active and healed lesions.

The diffuse type of reaction, already described, was frequently observed in the pericardium over the anterior surface of the pulmonary artery. In four instances, Aschoff bodies were present in the inflamed pericardial tissue overlying the artery. The vasa vasorum of the pulmonary artery in this vicinity were frequently thickened and at times

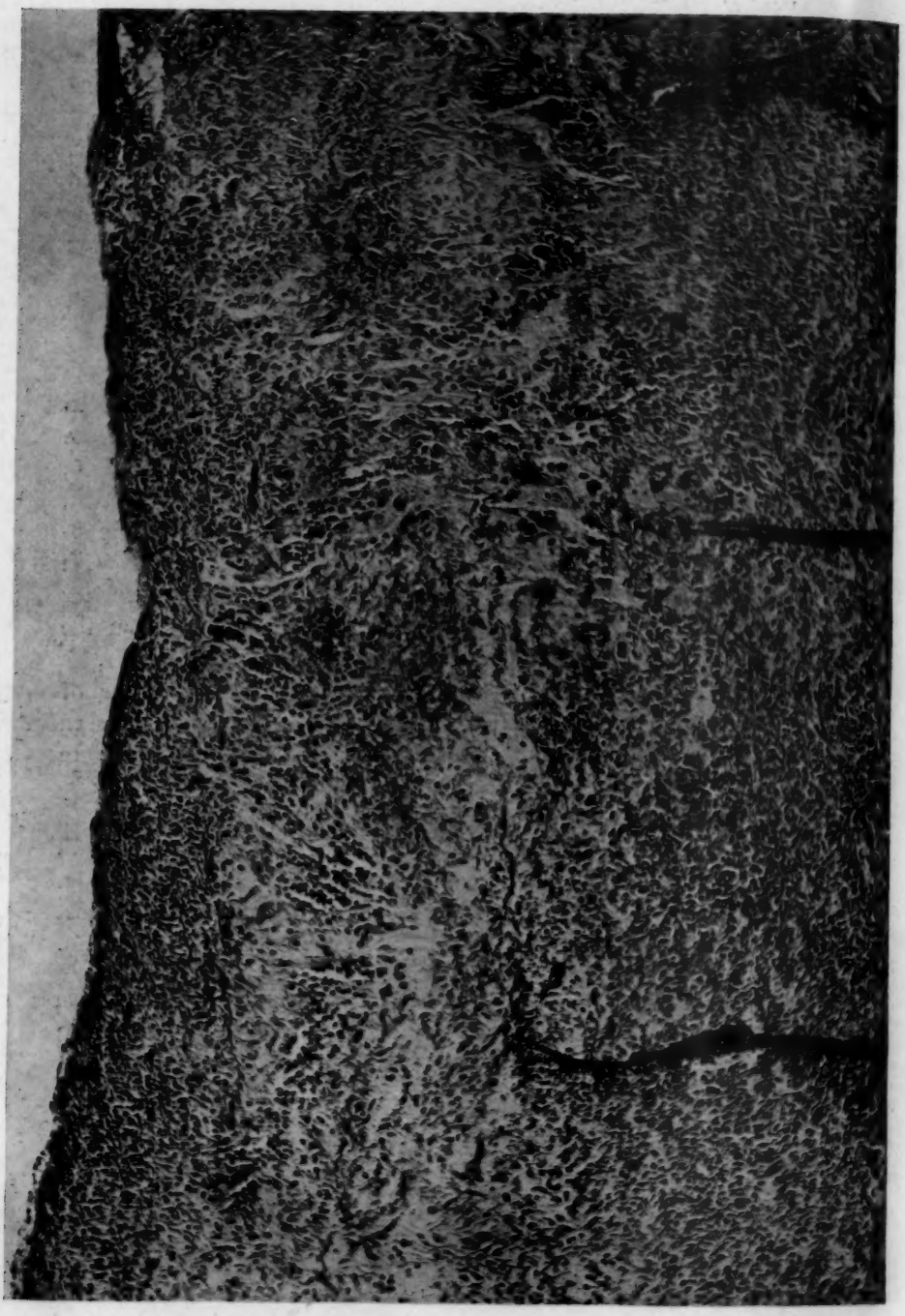

Fig. 3 (autopsy 5,822).—Pulmonary artery. Necrosis of intima, diffuse cellular reaction and scarring in the media. Penetration of parallel rows of cells from the subintimal into the deeper layers of the media.

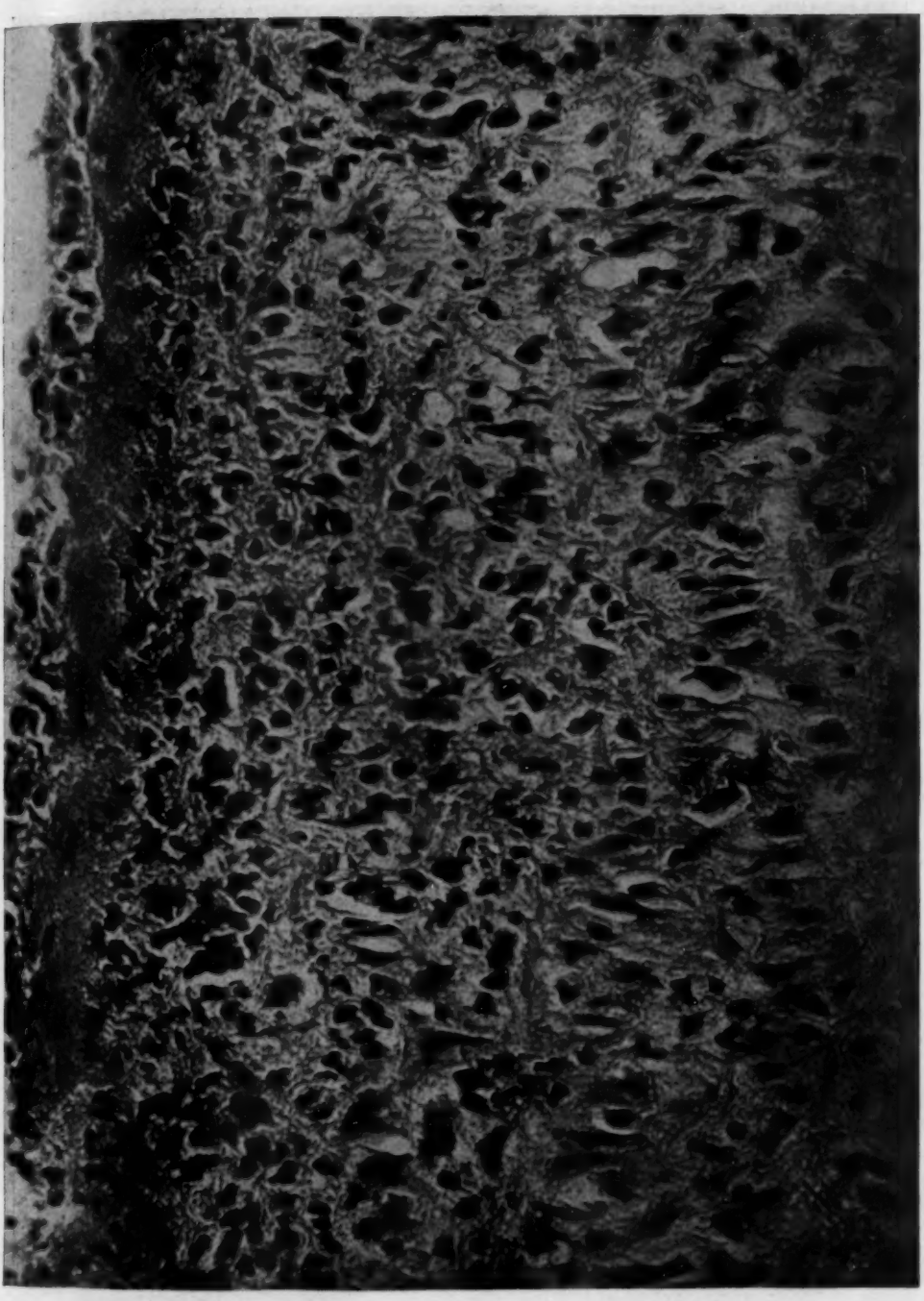

Fig. 4 (autopsy 5,822).—High power photomicrograph of inner layers of pulmonary artery. Details of intimal necrosis and diffuse extensive cellular infiltration should be noted.

Fig. 5 (autopsy 5,822).—Pulmonary artery. Diffuse reaction extending throughout all three coats. The perivascular collection of Aschoff cells about the nutrient vessels of the media should be noted particularly.

showed cellular infiltration which occasionally was sufficiently intense to result in partial or complete obliteration of the lumen.

C. Pulmonic Valve.—As many previous observers have noted, gross lesions of conspicuous size were uncommon in the pulmonic valve. In six of the twenty-four cases in which there was active rheumatic infection, verrucae were seen on the pulmonic valve, but in four of them the lesions were minute and inconspicuous, consisting of a few scattered vegetations on one or more cusps of the valve. In the other

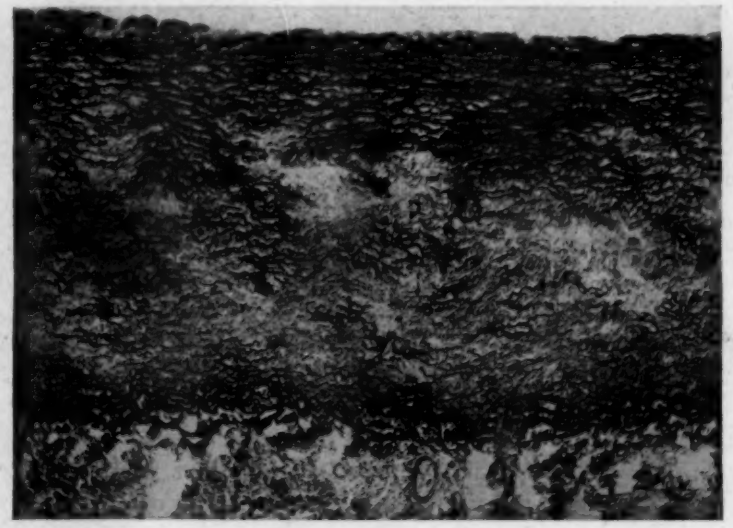

Fig. 6 (autopsy 5,698).—Pulmonary artery. Pronounced scarring of the media with disruption of the elastica (Weigert's elastic tissue hematoxylin; van-Gieson's stain).

two cases, however, a continuous row of fresh, translucent, pearly gray, verrucose vegetations (about 0.5 to 1 mm. in diameter) was seen along the line of closure of all the cusps. These verrucae were smaller than those usually seen on the mitral and aortic valves.

A systematic histologic study of this valve revealed an unexpected frequency of pathologic changes, i. e., in fourteen of the twenty-four active cases. In some of these, the valvulitis was limited, extending into the valve from its base for a distance of a third or a half of the length and ending usually where the valvular blood vessels [11] in this area terminated. In most instances, the valvulitis was associated with a diffuse reaction in the musculo-arterial junction, or at the base of the valve. The infiltrations in the valve were usually diffuse and

not specific, consisting of plasma cells, lymphocytes, large and small mononuclear cells and occasional polymorphonuclear leukocytes. In three cases, Aschoff bodies (fig. 7) were found in the pulmonic valve, and in other instances subintimal collections of the typical large cells were seen in the Aschoff nodule but not arranged in the characteristic nodular structure.

In a study of thirty cases of nonactive rheumatic cardiac disease, we occasionally found scarring and small lymphocytic foci in the musculo-arterial junction and fibrosis and slight disruption of the elastica in the media of the pulmonary artery. These changes, in all probability, represent the healed stage of the lesions found in the active cases.

D. Aorta.—For the sake of comparison, we studied the aorta, aortic ring and valve in the rheumatic cases by the same methods which we employed for the pulmonary artery and valve. The most conspicuous changes were those seen at the fibrous ring of the aorta, which was more often involved than the corresponding area of the pulmonary artery. A similar inflammatory process, usually of the diffuse type, was present in twenty-two of the twenty-four cases of active rheumatic infection, an incidence of 90.7 per cent. The intimal lesions present in four cases in the aorta were more striking than in the pulmonary artery, and resembled closely those described by Pappenheimer and von Glahn.[6] In five cases the media of the aorta was the seat of an active inflammatory reaction, and in each instance the reaction was diffuse. Aschoff bodies were not found in the wall of the aorta. On the whole, in our series, changes in the media of the aorta were less intense and less characteristic than those observed in the pulmonary artery. In one case, the endothelium of the root of the aorta and the valve in close proximity at the sinus of Valsalva was the seat of a typical verrucose endocarditis. This large flat verrucose lesion extended from the root of the aorta upward along the arterial aspect of the aortic valve. Deeper in the root one discerned a diffuse reaction with lymphocytes and various types of wandering cells.

CONTROL GROUP

As previously state, two groups of cases were selected: (1) cases of pericarditis unaccompanied by rheumatic cardiac disease, and (2) routine autopsy cases in which neither pericarditis nor rheumatic cardiac disease was present. These control groups were studied in the same way as the rheumatic cardiac group.

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11. Kugel, M. A., and Gross, L.: Am. Heart J. **1**:304, 1926.

Fig. 7 (autopsy 5,456).—Pulmonary valve. Extensive diffuse interstitial valvulitis, showing diffuse reaction and focal collection of cells (Aschoff bodies).

The thirty-one cases of nonrheumatic pericarditis included instances of bacterial (pneumococcic, streptococcic, staphylococcic and tuberculous), uremic and localized reactive pericarditis accompanying myocardial infarction due to coronary thrombosis, but not in any of these were changes found in the pulmonary artery which were at all comparable to those seen in the rheumatic cases.

The pulmonary artery was investigated in seventy-five routine postmortem examinations, in which neither pericarditis nor rheumatic cardiac disease was found. The autopsy material was most varied but unfortunately did not include cases of acute infectious diseases such as typhus fever, typhoid fever, scarlet fever and diphtheria. Such a group would be valuable for control examination, since these infections might conceivably produce lesions in the artery more or less resembling those found in rheumatic cases.

In no instance in the control groups were lesions found that simulated in degree and type those found in the active cases of rheumatic cardiac disease. The vulnerable musculo-arterial junction did not show more than a slight amount of fibrosis in certain instances. The pulmonic valves were entirely free from inflammatory disease. The pulmonary artery at times showed changes in the elastica of the media, which were relatively insignificant, except in a few cases of nonrheumatic pericarditis in which there were conspicuous scars in the media. In a number of instances the nutrient arterioles were thickened, especially in cases of atherosclerosis. Occasionally there were scattered, isolated, irregular, small collections of mononuclear and plasma cells or lymphocytes in the media of the artery.

COMMENT

Pending the discovery of the etiologic factor of rheumatic fever, the only criterion which establishes the rheumatic nature of a pathologic process is the Aschoff nodule. Unfortunately, these nodules are not always found, so that many cases which, apparently, are definitely rheumatic cannot be proved to be so. It is, therefore, all the more desirable to add as many facts as possible to the knowledge of the pathologic manifestations of the disease, so that it may be possible eventually to identify definitely those cases in which the Aschoff bodies are absent.

In the pulmonary artery as well as in the aorta, we were confronted with two distinct types of lesions, a diffuse cellular reaction and the perivascular Aschoff cells. In four instances we found the specific nodules in the epicardium of the pulmonary artery, and in three instances in the pulmonic valve.

As to the pathogenesis of the involvement of the pulmonary artery, there are three possibilities: (1) a direct effect on the intimal wall, (2) invasion by way of the vasa vasorum [6] and (3) invasion by direct extension from the inflamed pericardium. The presence of a diffuse cellular reaction in the intima of the artery, arranged at times in palisade formation and also in rays radiating inward, with concomitant thickening of the intima, would suggest direct infection of the intima by way of the lumen, whereas the presence of focal perivascular cellular collections and, in the healed stage, scars due to necrosis of the muscle with disruption of the elastica, points to involvement by way of the vasa vasorum or by contiguity from the pericardium. In the five instances of lesions in the pulmonary artery there was a concomitant pericarditis. However, there were six instances of rheumatic pericarditis and thirty-one cases of nonrheumatic pericarditis in which there were not any changes in the pulmonary artery.

We found as the most frequent lesion a diffuse infiltration of the musculo-arterial junction, which at times contained the characteristic multinuclear cell found in the Aschoff nodule. This diffuse reaction, while associated with rheumatic cardiac disease cannot yet be considered specific, since we have been unable to study the pulmonary artery in such infections as typhus fever, typhoid fever, diphtheria and the exanthemas. From a perusal of the literature [12] it appears that inflammatory changes in vessels may also occur in these conditions. It would be instructive to compare these changes with those in rheumatic fever.

We feel with Pappenheimer and von Glahn [6] that the diffuse reaction, in spite of the fact that its component cells are not specific in kind, plays an important rôle in the response of the body to the rheumatic infection, though the value of this reaction for the diagnosis has not yet been established.

The frequent occurrence of lesions in the musculo-arterial junction affords two interesting speculations, one clinical and the other pathologic. The frequent involvement of the ring of the valve, in both aortic and pulmonic segments, may account for some of the transient auscultatory signs often heard during the course of rheumatic fever. In the pathogenesis of valvulitis, it is easily conceivable that the inflammation, which has started in this vulnerable place, may extend up into the valve and there set up a corresponding reaction. The verrucose deposits on the pulmonic valve, except in two instances, were inconspicuous. Such lesions might completely heal without producing any appreciable gross deformity of the valve, which fact would also account for the infrequent observation of macroscopic involvement of the pul-

12. Martin, H.: Rev. de méd. **1**:383, 1881; **3**:103, 1883. Barié, E.: Rev. de méd. **4**:1, 1884. Romberg, E.: Deutsches Arch. f. klin. Med. **48**:369, 1891.

monic valve in rheumatic cardiac disease. Healing in the musculo-arterial junction, valve and artery may be evidenced by the observation of fibrous tissue and small foci of round cells.

In the thirty cases of chronic rheumatic heart disease without evidences of active infection, we occasionally found scarring and small lymphocytic foci in the musculo-arterial junction and fibrosis and slight disruption of the elastica in the media of the pulmonary artery. We did not observe any of the acute lesions seen in the active cases.

SUMMARY

A systematic study of the pulmonary artery, valve and musculo-arterial junction was undertaken in twenty-four cases of active rheumatic infection of the heart. In five of these, histologic examination of the artery revealed active inflammatory changes consisting, on the one hand, of diffuse cellular infiltration in the intima and subintimal layers of the media and, on the other, of a focal perivascular collection of cells comparable to the Aschoff nodules in the myocardium. These changes were similar to those described by Pappenheimer and von Glahn in the aorta. In two instances the intimal changes were sufficiently widespread to produce small macroscopic lesions on the inner surface of the vessel. In two cases the disruption of the elastic media was almost as conspicuous as in syphilis.

In fourteen of the active cases, the pulmonary valve was the seat of an interstitial valvulitis which involved a part or the whole of the valve, and in three of these the diffuse reactions was accompanied by the Aschoff bodies in the substance of the valve. Verrucae were present in six cases, but these were inconspicuous in all but two.

The musculo-arterial junction was the seat of active inflammation in seventeen of the twenty-four cases, or in 70.9 per cent. The more frequent type of reaction was of the diffuse variety, but Aschoff bodies were also present in a few.

In a comparative study of the aorta, its root and valves, the fibrous ring of this vessel which corresponds to the musculo-arterial junction of the pulmonary artery, was involved in twenty-two, or 90.7 per cent, of the twenty-four cases of active rheumatic heart disease. In four instances there were gross lesions in the intima, and these were more extensive in the aorta than in the pulmonary artery. Diffuse inflammatory changes were found in the wall of the artery in each of these cases. Aschoff bodies were not seen.

As controls, we studied thirty-one cases of nonrheumatic pericarditis and seventy-five cases in which neither pericarditis nor rheumatic cardiac disease was present. In none of the control cases were lesions found which could be confused with those seen in the pulmonary artery, valve and musculo-arterial junction in cases of active rheumatic cardiac disease.

Laboratory Methods and Technical Notes

EXAMINATION OF SPUTUM FOR ACID-FAST BACILLI*

H. C. Sweany, M.D., Chicago

The number of cases in which the sputum is analyzed at the Chicago Municipal Tuberculosis Sanitarium has increased to such an extent that approximately 250 specimens are examined daily. In order that this may be done it has been necessary to systematize the work rigidly, and I feel that the results obtained are worthy of being reported.

SUPPLIES AND EQUIPMENT

1. Glassware:

   Round sputum bottles of 1 ounce (28.35 Gm.) capacity with wide mouth and measuring 10 cm. over all..... 5,000
   Plain 15 cc. centrifuge tubes..... 1,000
   Glass slides measuring at least 1 by 3 inches (2.5 by 7.6 cm.)..... 2,000
   1 gallon earthen jars with handles..... 24

2. Reagents:

   "Chromegemesh"—prepared from commercial sulphuric acid and potassium bichromate—made fresh every week and replenished as it becomes inactive or is all used up.
   Washing soaps and powders.
   Cooper's carbol-fuchsin stain (3 per cent sodium chloride in Ziehl-Neelsen carbol-fuchsin [1]).
   Acid alcohol (5 per cent nitric acid in 95 per cent alcohol).
   Methylene blue (Loeffler's diluted to about 30 per cent strength for counter stain).
   Sodium hydroxide (3 per cent in a siphon bottle with rubber tube and pinch cock).
   Compound solution of cresol or "izal" disinfectant—sufficient quantity.

3. Apparatus:

   4 microscopes, equipped with fluorite lenses, good substage lamps and mechanical stages.
   3 staining baths, Corper's [2] or Sweany's.[3]
   1 large international type 24 head shaking machine.
   1 16 head centrifuge, international type.
   1 incubator.
   1 autoclave.
   12 galvanized trays 6 inches (15.2 cm.) deep by 8 inches (20.2 cm.) wide.
   Platinum loops, grease pencils, labels, slide boxes, etc.

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* From the Research Laboratories of the City of Chicago, Municipal Tuberculosis Sanitarium.

1. Cooper, F. B.: Modification of Ziehl-Neelsen Staining Method for Tubercle Bacilli, Arch. Path. **2**:382 (Sept.) 1926.

2. Corper, H. J.: A Clinical Electrical Fixing and Hot Staining Apparatus, J. A. M. A. **65**:420 (July 31) 1915.

3. Sweany, H. C.: J. Lab. & Clin. Med. **10**:929, 1925.

PREPARATION OF GLASSWARE

All glassware is placed in earthen jars, covered thoroughly with fresh and active "chromegemesh" and allowed to stand forty-eight hours. The solution is then poured off, and the glassware is washed in hot tap water and afterward in distilled water, and dried in a dryer at 220 C. for thirty minutes. The washing in distilled water may seem a needless refinement, but no salt sediment remains on drying as is found after the tap water washing. When the glassware is used again, it is first placed in the trays, covered with compound solution of cresol or "izal" and autoclaved. After this the water tap is turned on over them and all the disinfectant is removed. They are again washed in soap and water, placed in earthen jars and treated as described previously. All faulty or etched pieces are discarded.

COLLECTION OF SPECIMENS

In this part of the work the dispensary physicians and nurses cooperate. The nurses label the bottle with all necessary data and collect the specimen

Results of Counts of Tubercle Bacilli Per Field After Twenty and Forty-Eight Hours' Incubation

No. 5 Incubation Period		No. 6 Incubation Period	
20 hours	48 hours	20 hours	48 hours
9	23	3	11
12	18	2	2
8	13	3	6
9	21	4	3
23	13	2	6
17	25	3	6
15	9	5	12
14	10	2	9
18	10	3	14
15	9	4	2
140	151	31	71

directly from the patient or leave the bottle at his home with proper instructions. When the specimen is brought in, the cork is pushed in tight, the label is secured by a rubber band and a triplicate report blank filled out and wrapped around the bottle. The bottle is then placed in a cell in the special carrying case made for that purpose. These specimens are picked up the next morning by a responsible messenger and delivered to the laboratory in good condition by 1 o'clock. Rarely does a cork come out if it is properly pressed in, and even if it does the safety trays will hold it in place.

THE SYSTEM OF EXAMINATION

When the specimens are received at the laboratory, the report blanks are removed from the bottles, checked against the name on the bottle and both bottle and blank are given the same number with a wax pencil. The specimens are then placed on trays, covered with cheesecloth, and incubated for from eighteen to twenty-four hours in the incubator. This process, instead of decreasing the number of bacilli, shows a greater number than is found by direct

treatment. Many times, the actual count is nearly double. After three days, however, the bacilli slowly disappear. Much of the mucus and cellular material is destroyed by incubation.

The result of a count of tubercle bacilli per field after twenty and forty-eight hours' incubation is given in the table. Incubation, therefore, increases the number of organisms in forty-eight hours. Figures 1 and 2 are a comparison of this method with the antiformin method. The former concentrates more and leaves less débris.

If a bottle is too full of sputum, a cork with an opening in it is exchanged for the first cork, and the whole tray is covered with cheese cloth and fastened so that the corks will not blow out. After removal from the incubator the bottles are filled with approximately as much again of 3 per cent sodium hydroxide making a final dilution of 1.5 per cent sodium hydroxide. The bottles are then placed in the shaking machine and shaken for from fifteen to twenty minutes, after which they are incubated for thirty minutes; finally the contents are poured into the centrifuge tubes bearing the same number as the bottles, and centrifugalized at about 3,000 revolutions per minute for eight minutes. The supernatant liquid is then poured off, and the drops of liquid are allowed to drain completely. The top of the sediment is scooped off gently with a platinum loop and smeared thin on a slide bearing the same number as the centrifuge tube. Should the amount of sediment be too small, an albumin fixative may be used to fix it and make the specimen more visible. Should there be too much mucoid material still present, as happens occasionally, the sediment is treated with 5 cc. of 3 per cent hydrochloric acid, stirred and centrifugalized again for five minutes. This treatment reduces the sediment again over half. Throughout the whole process every specimen, tube and slide is always kept in order. This is a safety check. The slides are placed in a rack and fixed over the electric bath, after which they are stained for from eight to ten minutes by moderate heating. The slides are then washed in tap water in a special tray, by means of a rubber tube attached to a water faucet. Decolonization is carried on to completion (from five to fifteen minutes), and the washing is repeated, the counter stain applied and a final washing given. The slides are dried over the bath and are then examined until they are found to be positive or for six minutes if they are negative. It has been found by experience that in six minutes about 500 fields will have been covered, or about one thirtieth of the average slide. This is the equivalent of two whole slides by the direct smear method because the sputums are concentrated from 50 to 150 times. It is rare that at least one bacillus is not found in the first six minutes if the slide is positive. If one bacillus is found then a search is made until more are found. It is almost a law that one does not find one or two tubercle bacilli alone on a slide. It is possible, but with the method of concentration described here it is almost always probable to find ten or more bacilli. Once in a long time as few as four or five are found. If fewer than that number are found, the material is reexamined until more bacilli are found or the result is only suggestive. One, two or three bacilli, therefore, are considered only suggestive and a signal for repeated examinations. In 20,000 examinations made so far only once has a report of less than four per field been made, so that it has been necessary to disregard such an observation of acid-fast bacilli as an error or accidental. Five times, from four to ten per slide have been found. After a thorough search and repetition of the examination, the specimen usually falls automatically into the positive or negative group, and the probability of error is small.

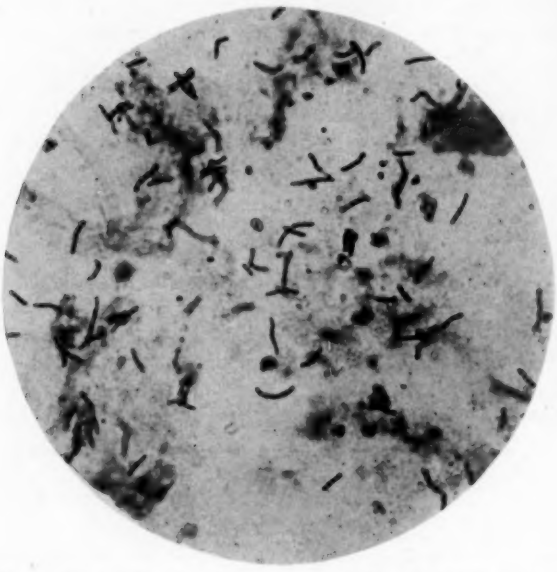

Fig. 1.—Photomicrograph of stain smear of sediment from sputum after treatment by the antiformin method; × 1,200.

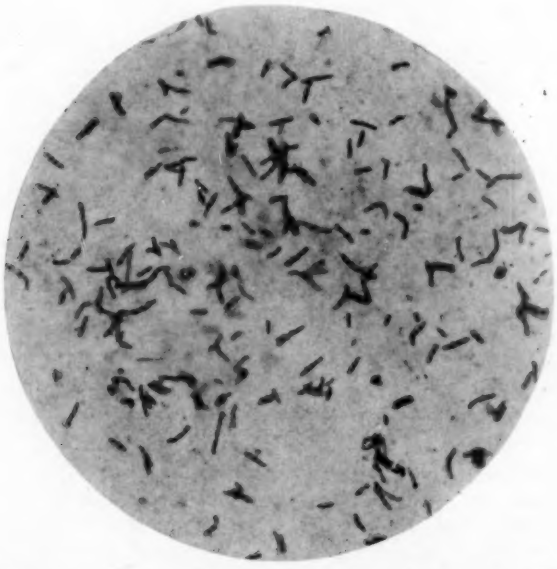

Fig. 2.—Same sputum as in figure 1 treated by the new method. The chief difference is the less amount of débris and the greater number of bacilli; × 1,200.

After the slides are found to be positive, they are registered in a special record book, labeled and filed for future reference. The reports are then marked, mailed or delivered directly to the proper place, after a duplicate is filed in the records. Within three days from the time the sputum is collected, the reports are received, although some may come from places thirty miles distant.

COMMENT

It must be emphasized that these reports are only for acid-fast bacilli; yet I doubt if one encounters many acid-fast bacilli in the sputum other than those causing tuberculosis. It is possible that of the 100,000,000 tuberculous lesions in the country alone that are present in clinically negative cases, occasionally one will give off a few organisms that are found easily by the method described. A clinical examination cannot be made in the laboratory, but a positive result in the "clinically negative" case is at least a sign of warning.

It must be pointed out also that there are stages of tuberculosis both before the onset and after recovery, in which it is physically impossible to elicit signs which are audible through 2 inches (5 cm.) of lung tissue, pleura, bone and muscle; yet bacilli will be present. Furthermore, clinicians have not been able to find nearly all the open cases of tuberculosis, so that a positive sputum in a "clinically negative" patient may be a "latent" tuberculous lesion; it may be the onset of a case of tuberculosis that will be evident in time; it may be the closing out of an undetected tuberculosis, or it may be only a flare-up of a lesion too small to produce clinical signs. Such things are more than possible; I have actually seen them in my postmortem work.

Another objection may be raised against Cooper's modification of the Ziehl-Neelsen technic. On a large variety of specimens this method has been found consistently negative in negative specimens and clearly positive in the others. It is a distinct advance in staining for tubercle bacilli.

Such a system, as has been outlined here is a step forward in the early diagnosis as well as the early recognition of tuberculous patients. Accurate comparative studies are being made on the various phases of this method, and these will be reported later.

A METHOD OF MOUNTING BRAIN SECTIONS*

CYRIL B. COURVILLE, M.D., LOMA LINDA, CALIF.

Many methods which are more or less time consuming, and not always satisfactory as to end-result have been suggested for the mounting of serial sections of the brain. After some thought I devised a method which is well worth the time and effort expended. It is adaptable either for sections of the normal brain or for demonstrating the location and extent of lesions in brain tissue. Sections may be frontal,

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horizontal or saggital, depending, of course, on the purpose for which the specimen is mounted. They may be cut as thin as 1 cm. or even less.

In the hope that others may be interested in a plan for mounting sections of the brain in such a way that they will be of value in demonstration and study in medical schools, I shall describe my method.

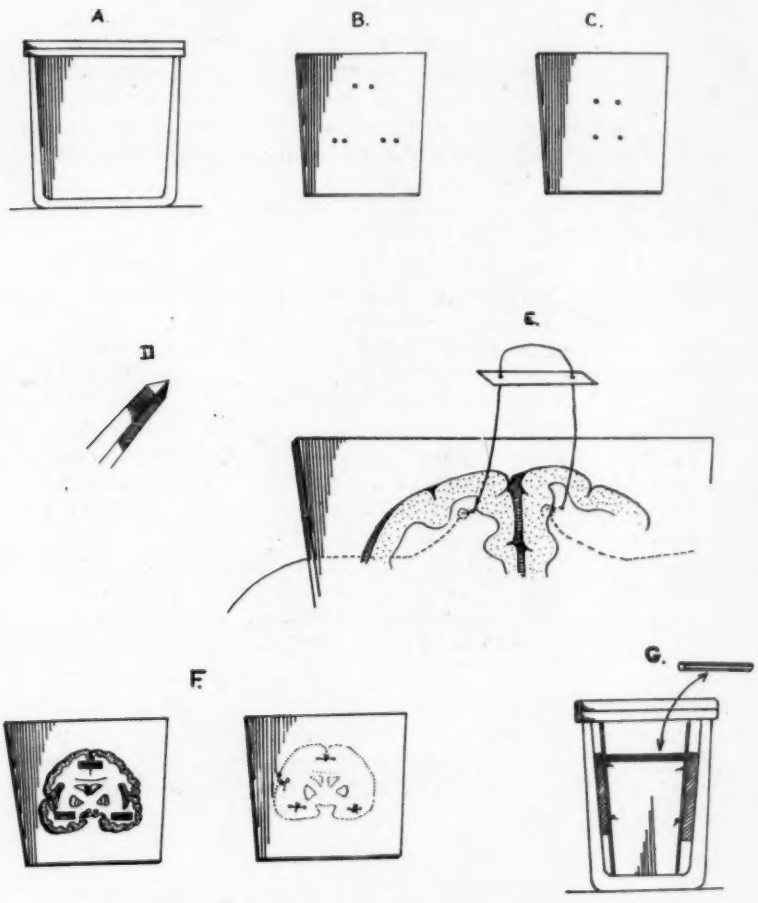

Fig. 1.—*A*, American Museum jar no. 4; *B*, glass plate with six perforations for large sections; *C*, glass plate with four perforations for small sections; *D*, bit made of three cornered file, used for boring holes in glass plates; *E*, sketch showing method of fixing sections to glass plate; *F*, obverse and reverse of glass plate with brain section in place; *G*, side view of jar showing peg between the plates.

METHOD

The sections are mounted on glass plates which may be cut from ordinary window glass to fit the internal measurements of the display jar. Black or white glass may be used for these plates if preferred. I find size 4 of the American Museum jars to be the most generally satisfactory. It is well to cut these

plates slightly smaller than the inside measurements, for the jars are not absolutely uniform in size. In this way, measuring and cutting for each individual jar is avoided.

After the plates have been cut, they are perforated in four or six places depending on the size of the section to be mounted (fig. 1, *A, B, C*). This is accomplished by drilling with a brace and bit. The bit is made from an ordinary triangle file, the blunt end of which is ground to a point on an emery wheel (fig. 1, *D*). The glass is treated with a saturated solution of camphor gum in turpentine before and during the drilling. Great care must be used in drilling,

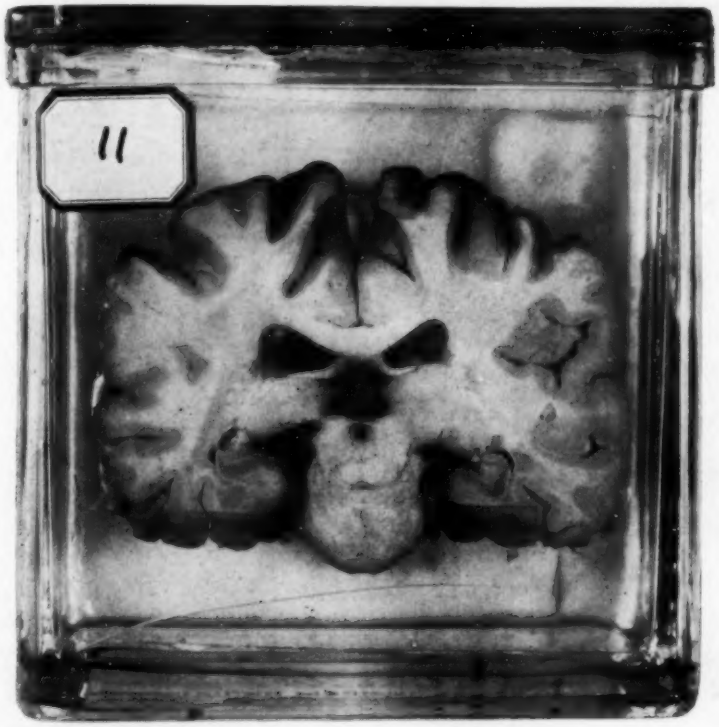

Fig. 2.—Brain section mounted in jar.

for too much pressure on the glass will break it. Two such plates are prepared for each jar.

Serial sections are mounted on the prepared glass plates as follows: The section is placed on the glass in the desired position. A gray silk thread on a fine needle is passed from below through one of the holes in the glass, through the tissue and then through a fine strip of celluloid which has been perforated previously in two places corresponding to the holes in the glass. The procedure is then reversed, the thread being passed through celluloid, tissue and the perforation in the glass (fig. 1, *E*). The two ends are firmly tied, the celluloid strip preventing the thread from cutting the tissue. This strip is invisible when the section is immersed in the solution, and the thread shows but little, comparing closely to the brain tissue in color. I have made it a practice to fasten the speci-

men near the vertex about half an inch (1.27 cm.) on either side of the median fissure, and laterally by the temporal areas. This is sufficient to secure the specimen (fig. 1, *F*).

The sections are then mounted in serial order in a series of jars, one on either side. The specimen is firmly pressed against the side of the jar, and its position is maintained there by a tightly fitting wooden peg which is inserted between the two plates (fig. 1, *G*). The finished sections when mounted and immersed in solution show all the details clearly, and the section appears to be suspended in the jar, the glass plate support being invisible (fig. 2).

We have been using formaldehyde for hardening specimens, but if fresh brains are used it would be advisable to adopt the following procedure:

1. The fresh brain is immersed in Jore's solution for three days until it is partially hardened.

2. The sections are cut as previously suggested. They are replaced in Jore's solution for five days more, or longer if indicated, until the specimen is perfectly preserved.

3. The sections are mounted on glass plates and sealed in demonstration jars in Klatz' solution.

Thus, the color is perfectly preserved, and the entire plan presents a most perfect method for the demonstration and study of brain sections.

A card has been prepared on which there is a fully labeled outline drawing corresponding to each mounted section. These cards are mounted permanently on a square of pressed cardboard and covered with a piece of celluloid, the three being fastened together in a stapling machine (Challenge Riveter). The cards are labeled with a key number similar to that on the jar and filed when not in use.

Thus far my efforts have been confined to brain tissue, but I do not see any reason why the plan could not be adapted to any type of anatomic or pathologic tissue in which the thickness or fragility of the specimen prevents its being mounted in the ordinary way.

General Review

THE PRESENT STATE OF KNOWLEDGE REGARDING EPIDEMIC ENCEPHALITIS

THIRTEENTH MELLON LECTURE *

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On a subject in which there are so many unanswered problems and in which the accumulation of facts has been far more rapid than their correlation, it is necessary for the purposes of a single lecture to select those particular phases which seem to be of fundamental significance. Moreover, thorough general summaries of the knowledge concerning epidemic encephalitis have been published in the *British Public Health Reports*,[1] by Smith in the publications of the United States Public Health Service,[2] by Gottstein,[3] by Flexner,[4] by van Boeckel, Bessemans and Nelis,[5] by Levaditi,[6] by MacNalty [7] and others. A large number of thorough clinical treatises of the disease have also been published by capable neurologists throughout the world. I have chosen, therefore, to confine myself largely to those aspects of the problem which deal with its characteristics as an infectious disease. I am fully aware that even in this limited part of the subject I can give final answers to few of the fundamental questions involved, but it is useful in a problem as difficult as this to collect and appraise evidence from time to time in order to discriminate clearly between things that may be accepted as facts, those which are probable and those which—being certainly wrong—may be disregarded in the guidance of future investigation.

* From the Department of Bacteriology and Immunology, Harvard University Medical School.

* Read before the Society for Biological Research, School of Medicine, University of Pittsburgh, May 10, 1928.

1. Discussion of Encephalitis Lethargica, Brit. M. J. **2**:488, 1918.
2. Smith, H. F.: Pub. Health Rep. **36**:207, 1921.
3. Gottstein: Ergebn. d. Hyg. Bakt., Imm. Forsch. Weichardt. **5**:394, 1922.
4. Flexner, S.: Epidemic Encephalitis and Allied Conditions, J. A. M. A. **81**:1688 (Nov. 17) 1923.
5. Van Boeckel, L.; Bessemans, A., and Nelis, C.: L'encephalite lethargique, Brussels, Nossent & Cie, 1923.
6. Levaditi, C.: L'herpès et le zona, Paris, Masson & Cie, 1926.
7. MacNalty, A. S.: Epidemic Diseases of the Central Nervous System, London, Faber & Gwyer, 1927.

The sporadic manner in which the disease is appearing in many parts of the world today, and the protean clinical forms in which it appears have sometimes masked the true nature of the disease in recent reports, and have made it desirable again to consider whether one is dealing with a condition that seems to be caused by an extraneous virus—in other words, a transmissible invasive organism—or whether its etiologic basis is not more likely a toxic degeneration, secondary to other causes. The experimental problem, moreover, is probably rendered particularly difficult at this time, since it is probable—as stated by an experienced clinician not long ago—that practically all the cases now under observation in most clinics are from one to two years old, few if any acute ones being available for study.

CLINICAL FEATURES OF ENCEPHALITIS

It is therefore more than likely that most of the cases which come to the attention of the general practitioner at interepidemic times like the present have already passed the early acute stages during which those features which characterize an infection have disappeared, and the disease has already assumed a chronic form. That this is equally true of the similar disease of poliomyelitis, in spite of the fact that in general physicians are far more familiar with this condition than with epidemic encephalitis, is apparent. For an appraisal of the significant features of the onset of encephalitis, therefore, it is advisable to examine the reports of cases observed during epidemics, when physicians were expecting them and were more likely to scrutinize all obscure conditions with this disease in mind. Smith, of the United States Public Health Service, in summarizing the onset, says that in about 30 per cent of the cases studied by him the condition appeared suddenly, with headache and fever, and that fever was the most constant single early symptom. This corresponds with the report of Hall [8] in a discussion of the cases that appeared in England in 1918. In von Economo's [9] early cases, the onset was usually accompanied by fever, chilliness—which he describes as influenza-like—nausea, headache and signs of meningeal irritation. In milder cases, these earmarks of an infectious invasion were often absent or negligible, but they were sufficiently frequent in the more severe cases to remove any question about their importance. In von Economo's observations also, the spinal fluid was often under pressure, and though it contained relatively few cells—an average of from 10 to 20 per cubic millimeter, rarely as many as 100—the number of polymorphonuclear leukocytes was relatively high. The blood leuko-

8. Hall, A. J.: Brit. M. J. **2**:461, 1918.

9. Von Economo: Wien. klin. Wchnschr. **30**:581, 1917; Die Encephalitis Lethargica, Leipzig, Franz Deuticke, 1918.

cytes are apparently not appreciably changed, the reports of various observers agreeing with the series cited by Smith in which, in 51 per cent, the leukocytes numbered less than 10,000 per cubic millimeter; in the remainder, they ranged between 10,000 and 15,000.

It is particularly important to study the reports of the clinicians who saw the disease when it first appeared, since there is a great deal of information which indicates that as epidemics have decreased, clinical features have changed. The earlier cases of encephalitis occurring in 1917, 1918 and 1919 were chiefly epidemic, with ophthalmic symptoms rather prominent. In 1922 and 1923, according to Stallybrass,[10] the epidemic type gave way to one in which there was extreme restlessness, often delirium with terror, and other conditions, and a greater liability to relapse. At the same time, the mortality also increased, rising by steps in England from 12 per cent in 1920 to about 40 per cent in 1923.

After the onset, the many clinical varieties—which are so manifold that Tilney and Riley [11] divide the types into eight subdivisions, a state of affairs due, of course, to the irregularity of the distribution of the lesions—offer little that particularly suggests an infectious nature. But if one analyzes the various reports of the manner in which the disease began, one finds in this malady practically the same type of evidence of an infectious onset that there is in poliomyelitis, in which the neurologic symptoms likewise develop, as a rule, after the fever and the initial generalized symptoms have subsided.

Pathologic Changes.—The pathologic changes in epidemic encephalitis are of particular importance in connection with this theme, because it is necessary to define pathologically not only the typical cases of primary epidemic encephalitis, but those forms of encephalitis which are clinically similar—indeed, indistinguishable from von Economo's disease—but in which there is a distinct clinical relationship to toxic injuries and infections such as measles, vaccination, chickenpox, whooping cough and lead poisoning—a relationship which will be discussed in a subsequent section. In encephalitis, the physician is confronted with a condition that may be either an actual infectious process or one due to toxic injury exerted on the brain by poisons absorbed and produced elsewhere. Perhaps the most striking single element is that of minute hemorrhage, which is peculiar in that it usually appears as though red blood cells had merely passed through the capillary walls without a preceding inflammatory reaction. Some pathologists—I have this idea from a tentative opinion expressed by Mallory and Parker—are inclined to suspect that the hemorrhage is the primary lesion, other things either being consequences of this or following in its train from the same injury that acts

10. Stallybrass, C. O.: Lancet **2**:922, 1923.

11. Tilney, F., and Riley, H. A.: Neurol. Bull. **2**:106, 1919.

on the capillary walls. There is a certain amount of cuffing of the capillaries and a moderate increase in the satellites around the nerve cells, a condition interpreted as neuronocytophagia. There is some edema, slight lymphocytic infiltration in the pia mater and a certain amount of endothelial proliferation. It is notable that in some cases that come to autopsy there is astonishingly little in the pathologic changes of the brain to account for the fatal disease. One of the characteristic things in epidemic encephalitis is the distribution, which here—in contrast to poliomyelitis—is more especially in the pons, the medulla and the optic thalamus, one of the most infiltrated areas being the substantia nigra. The spinal cord, if it shows any lesions, shows far less than in poliomyelitis. Nevertheless, the individual histologic lesions have considerable similarity to those in poliomyelitis, as far as infiltration of round cells around the capillaries and the increase of satellite cells are concerned. There is, of course, much less hemorrhage in poliomyelitis, although I have seen hemorrhages in monkeys inoculated with this virus; and in poliomyelitis there is a different distribution, of course, the mesencephalon being less affected than the cord. In most cases an experienced pathologist can distinguish between a case of encephalitis and one of poliomyelitis, but the lesions are sufficiently similar to offer occasional difficulties and at least to suggest a similarity in method of attack of the injurious agents.

In connection with the important problem created by the frequency with which encephalitis—clinically indistinguishable from the epidemic form—follows various other diseases, it is important in this place to compare as well as possible the lesions of these various conditions.

I have not had the opportunity to study cases of postvaccinal encephalitis, but Turnbull and McIntosh [12] have made a careful comparison of such cases, both with poliomyelitis and with epidemic encephalitis. They say that epidemic encephalitis differs from postvaccinal encephalitis in extent of distribution, though they report two cases in which the distribution was similar. In histologic detail, they say that there are many resemblances but also differences, and they believe that these differences can be quickly determined by the use of the low power lens of the microscope. Most striking among these differences is a zone of softening often found around vessels in the white matter. But in summarizing, they say that histologically it is more likely that postvaccinal encephalomyelitis can be caused by the virus of epidemic encephalitis than by that of poliomyelitis.

The lesions in encephalitis following lead poisoning are similar to some extent to those in epidemic encephalitis in that there are hemorrhages and cuffing of blood vessels and—since there is a definite toxic

12. Turnbull, H. M., and McIntosh, J. A.: Brit. J. Exper. Path. **7**:181, 1928.

agent which can be determined as being present in the nervous system, a distinct suggestion presents itself in favor of the view which constitutes an important alternative of reasoning—in that epidemic encephalitis may be due to a toxic agent produced elsewhere in the body, which, like the toxins of tetanus and *Bacillus botulinus,* is absorbed from other portions of the body. This, of course, is in keeping—as will be seen—with the fact that the majority of workers have failed to recover any type of virus from the brains of patients suffering from this disease.

On the other hand, these lesions—so strongly suggestive of toxic injury—are still obviously similar to those found in poliomyelitis, not fundamentally different from those present in rabies, and in some of their features comparable to the lesions described by Wolbach [13] in the brains of animals with typhus fever—all of these diseases in which a demonstrable virus is present in the brain.

It may be justly held, therefore, that the pathologic changes in the brain in encephalitis, significant as they occasionally are, are not inconsistent with the presence of an invading agent, and—more than this—that they are indeed consistent with the assumption of the presence of a virus arousing the same general type of reaction as that in poliomyelitis or rabies. They are distinctly inconsistent, however, with the assumption of a bacterial process, and should have much weight in prejudicing one against the ready acceptance of a bacterial etiology by any of the various forms of cocci which have been described for both encephalitis and poliomyelitis. Such organisms cannot arouse a pathologic reaction of this kind, certainly not in acute cases and probably not even in the most chronic types, unless they have been completely metamorphosed, not only in their forms and sizes, but in their methods of attack on the tissues of the host, and when one assumes such metamorphosis, one is, of course, entering the field of speculation.

Epidemiology and Evidence of Transmission.—It is probable that the disease which, since von Economo's description, is recognized as a clinical entity had appeared in epidemic form in preceding centuries. In 1712, Biermer [14] studied an epidemic disease in Tübingen which was popularly known as "the sleeping sickness," since it was accompanied by somnolence and cerebral symptoms. Le Pecque de la Cloture,[15] in 1769, described a similar disease, which he speaks of as "Coma Somnolentum" and which was apparently associated with an influenza-like malady.

13. Wolbach, S. B.; Todd, J. L., and Palfrey, F. W.: The Etiology and Pathology of Typhus. The Main Report of the Typhus Research Commission of the League of Red Cross Societies to Poland, Cambridge, Harvard University Press, 1922.

14. Biermer, in Virchow: Spezielle Pathologie und Therapie, 1865, vol. 5 (quoted from von Economo).

15. Le Pecque de la Cloture, quoted from Lonquet: Semana méd. **12**:275, 1892.

Bassoe [16] mentions similar conditions described by Ozanam which occurred in Germany during the eighteenth century, in Lyons in 1800 and in Milan in 1802. Following the epidemic of influenza in 1889 and 1890, many cases were carefully described by Leichtenstern [17] which clinically appear to fall into the same category, and von Economo believes that the curious disease spoken of as "nona," of which there was a widespread epidemic during the early nineties of the last century in northern Italy, Switzerland, Dalmatia and Hungary, cannot be differentiated by the clinical descriptions from the disease which he observed in Vienna in 1917. The cases in Vienna appeared rather close together in point of time, so that von Economo obtained the impression of an epidemic distribution, and it is important for subsequent considerations to note that his cases preceded the concentration of cases of influenza of the first waves of the last great epidemic.

It is probable that Cruchet [18] and others saw similar cases at about the same time in France. In England, the disease unquestionably appeared during the first half of 1918, and in northern America, during the latter part of the same year. Between September, 1918, and May, 1919, 255 cases were reported in England. In America, they were more scattered, but between September, 1918, and May, 1919, Smith [2] collected 178 cases from nine states. These figures do not, of course, indicate any considerable degree of concentration of the disease in epidemic form, but they are sufficient, in view of the sudden appearance in various parts of the world, to indicate a probably infectious nature, which was von Economo's interpretation. The great epidemic which occurred in Japan during the summer of 1924, and which must for the present be regarded as the same disease, comprised 7,000 cases; 4,000 deaths resulted. Again indicating an epidemic nature is a seasonal concentration of cases during the months of January, February and March, and the distinct selection of an age group which is somewhat older than that prevalent for poliomyelitis.

Most important from the point of view of the determination of whether one is dealing with an infectious disease is evidence of transmission and group distribution. In considering this feature of any epidemic disease, it must be remembered that many factors may mask transmission from person to person, so that even in diseases like epidemic meningitis and poliomyelitis, in which the existence of an infectious agent is established beyond question, the tracing of a disease to its source is the exception rather than the rule. It is not often, even at the

16. Bassoe, P.: Epidemic Encephalitis, J. A. M. A. **72**:971 (April 5) 1919. Lonquet (footnote 15).

17. Leichtenstern: Influenza, in Nothnagel: System of Medicine, Vienna, Alfred Hölder, 1912.

18. Cruchet: Soc. méd. d. hôp. de Paris **25**:614, 1917.

height of epidemics of meningitis, or even with well established bacteriologic resources for determination of carriers and diagnosis that one can find any rime or reason in the manner in which cases are scattered about the community, and all that can be definitely determined in such a situation is the relationship between large carrier percentages and increasing case rates. In poliomyelitis, familial and other group distribution has been sufficient to indicate infection from contact and food, but—again—in the majority of cases the disease cannot be traced. This is obviously due to the existence of many carriers, the probable frequency of mild, undiagnosed cases, and the relative insusceptibility of a considerable proportion of the community—a matter to which I shall revert in a later section.

In epidemic encephalitis, it is probable that similar conditions prevail, and one cannot expect to find a great deal of evidence of direct transmission. Indeed, Bernard and Renault [19] say that from January to May, 1920, although there were more than 400 cases in France, there was not one case of direct contagion. MacNalty,[20] in 1919, and Netter,[21] in 1920, expressed the opinion that the disease was contagious. Parsons [22] analyzed the conditions in England and found a notable absence of outbreaks in schools, institutions and other public places in which individual cases had appeared, and pointed out the great rarity of evidence of personal contagion. Nevertheless, in a table quoted by MacNalty, Parsons recorded thirty-two instances of multiple infection, sixteen of which were familial cases, three of persons nursing infants with the disease and the rest of close association, which seemed unquestionable evidences of transmission. Similar meager evidence of infection from contact seems to have been the experience of most of those who have studied this phase of the disease. The following cases of apparent contagiousness are taken from MacNalty's book:

In Mansfield, three children of the same family came down with the disease in April and May. In Eastbourne, a boy, aged 13, and his sister, aged 12, who habitually slept in the same bed, came down within a few weeks of each other. In Weybridge, the disease afflicted a mother and son, both of them having had a preceding attack of influenza.

It is from an article by Stallybrass that I have taken the information concerning an outbreak in Germany occurring in 1920, in which, in an

19. Bernard, L., and Renault, J.: Bull. Acad. de méd., Paris **83**:470, 1920.

20. MacNalty, A. S.: Supplement to Forty-Eighth Annual Report, Local Gov. Board, London, 1919, p. 71.

21. Netter, A.: Bull. et mém. Soc. méd. d. hôp. de Paris **44**:441, 1920; **44**: 1030, 1920.

22. Parsons: Ministry of Health Reports of Public Health, 1922, no. 11, p 29.

asylum at Mülheim, there were twenty-eight cases with thirteen deaths, six of them nursing sisters and two physicians.

Kling and Liljenquist [23] describe an epidemic in Lapland, where from 7 to 45 per cent of the inhabitants of stricken villages were affected and where, in one instance, all the members of a single household had the disease. They also call attention to the fact that probably there were many mild, unrecognized cases by which the disease may have been spread. These observations from Lapland indicate a virulence so high that one would be inclined to question the correctness of the diagnosis were it not for the fact that these outbreaks are accepted as apparently authentic by observers as critical as MacNalty and Stallybrass.

Fyfe [24] describes four ambulatory cases occurring in the same school.

Stallybrass himself adds personal observations of similar significance. One of these is as follows:

A young man, aged 21, died of the disease on November 27. His sister, aged 13, had what was regarded as the relapse of chorea at Christmas, and by January 31 had developed diplopia, headache and fever. A boy, aged 15, who lived on the same street had a typical attack on December 26. On February 2, another case developed in the immediate vicinity, and on March 29 another child, aged 7, living on the same street, was found to have been suffering from encephalitis since February. This child had associated with the family of the first two patients.

From a report by Wilmer [25] which I have been privileged to see through the kindness of Benjamin White of the Massachusetts Department of Public Health, I have taken the following interesting case of transmission observed in connection with a typical case of encephalitis occurring in a young woman brought into a hospital in Massachusetts on the eighth day of the disease. Two weeks later, two nurses who had attended the patient came down with similar clinical symptoms, and two other nurses had an attack of a mild disease that was in general of a comparable nature. The report of this case was published by M. O. Pfister, and it was interesting to note that in the original case, in addition to symptoms referable to the cranial nerves, there was profuse salivation, a feature which is characteristic of herpes encephalitis in rabbits. Wilmer's report, incidentally, covers 623 cases, of which 162 are entirely reliable in regard to history, occurring in Massachusetts between 1921 and 1924.

Stallybrass also observed what he believes to have been mild, missed cases. He thinks that the development of the disease probably requires

23. Kling, C., and Liljenquist, F.: Compt. rend. Soc. de biol. **84**:521, 1921, and quoted from MacNalty.

24. Fyfe, L. L.: Lancet **1**:379, 1923.

25. Wilmer: Report to the Massachusetts Department of Health.

some other concomitant illness or injury affecting the nervous system, but his own cases did not show any relationship to influenza. His summarizing sentence is interesting in that he says: "Whatever the origin of the disease, we are witnessing an epidemic apparently affecting a virgin population—an epidemic which, further, appears to be in course of evolution."

Perhaps the most definite evidence of contagiousness is an outbreak which occurred in a girls' home at Derby, which was reported by MacNalty [26] in 1920. There were twenty-two residents in the institution. On August 14, two were taken ill, and by August 27, twelve had come down with the same disease; of these, five died. MacNalty studied the patients personally and determined the condition to be epidemic encephalitis. Cerebral tissues of the fatal cases were studied by Professor Turnbull, but it is strange that with this material McIntosh [27] succeeded in transmitting the disease to a monkey, which in my opinion throws some doubt on the true identity of this outbreak with the usual epidemic encephalitis. Although Turnbull and McIntosh found lesions in the inoculated monkey similar to those in epidemic encephalitis in man, they were not able to pass the virus from animal to animal. Their success in inoculating the first monkey may have some bearing on the case of two animals of my own, which will be discussed in one of the sections to follow. But however this may be, MacNalty himself, after sufficient experience with the disease, says that "the clinical features of the outbreak at Derby were so characteristic of epidemic encephalitis that little doubt can be felt that one was dealing with cases of the disease." He adds that two of the girls who recovered showed the typical sequelae. MacNalty also cites a few cases that are similar to the case of Pfister, in which there seems to have been direct evidence of transmission by close association, one of a nurse, aged 39, who came down with the disease nine days after she had taken charge of a patient.

There is considerable similarity, therefore, between this disease and both poliomyelitis and epidemic meningitis in regard to evidence of infectiousness. In all cases, the distribution of patients is a scattered and sporadic one at ordinary times, with occasional concentrations in time and place; even then, however, the instances that give clear evidence to direct transmission are exceptions rather than the rule. This, as I have already said, can be easily explained by the interpolation of variations produced by differences in susceptibility, carriers and mild cases, and there is no sound reason for questioning, it seems to me, that the same is true of epidemic encephalitis, since poliomyelitis and meningitis are definitely caused by a specific agent.

26. MacNalty, A. S.: Annual Report of the Chief Medical Officer, Ministry of Health, 1919-1920, appendix 7.

27. McIntosh, J. A., and Turnbull, H. M.: Brit. J. Exper. Path. **1**:89, 1920.

RELATIONSHIP OF ENCEPHALITIS TO OTHER DISEASES

One of the most important problems connected with epidemic encephalitis is its suggested relationship to other conditions. In a preceding paragraph I mentioned briefly that the early reports of Camerarius, of Le Pecque and of Ozanam indicated that the disease they described occurred in the train of catarrhal fever of some kind, which may have been influenza. After the epidemic of influenza in 1889, Leichtenstern and others described sequelae involving the nervous system, but while Leichtenstern's accounts clearly established the fact that encephalitic processes may follow severe attacks of influenza, neither the frequency of the cases nor the pathologic examinations make it possible to say with certainty that there was an encephalitis epidemic similar to the one I am discussing after the outbreak of influenza in 1889. Nona, which appeared in southern Europe, does definitely suggest the occurrence of a similar disease in 1890, but von Economo, in summing up this question, thinks that it is impossible to determine whether or not this disease—supposedly postinfluenzal—was truly epidemic encephalitis. During the last epidemic, the relationship with influenza was more definite. Von Economo's first cases, it is true, appeared before influenza had become prevalent to any considerable extent, but the connection seemed so definite to some writers that Gottstein expresses himself as believing that the relationship between influenza and epidemic encephalitis is comparable to that between syphilis and its late neurologic complications. Smith, in the monograph already referred to, analyzing American cases, states that in 46 per cent of the cases of encephalitis studied by him, the patients had a definite history of a preceding attack of influenza—which was considerably higher than the influenza rate in the general population. His point of view agrees with that of Gottstein, who suggests the name "Grippe Encephalitis." Other writers, notably Flexner, and more recently Jordan,[28] while admitting the association in time and concentration of influenza and encephalitis, come to the conclusion, which seems to me inevitable, that influenza may well be a contributory factor, but that the encephalitic process is a separate, pathologically distinct disease. Nevertheless, it must be borne in mind, especially in connection with the facts to be presented subsequently, that a great deal of evidence supports the view that the coincidence of influenza and encephalitis may be causally related, one disease rendering in some manner susceptible to the other. This point of view is further strengthened by the fact that it is not only influenza which is apparently associated with encephalitis, but many other injuries which may exert the same influence. Among these are vaccination, measles, varicella and smallpox—which have not infrequently led to a disease of the nervous

28. Jordan: Epidemic Influenza, Chicago, A. M. A. Press, 1926, p. 326.

system clinically similar to epidemic encephalitis—and occasional cases have followed pneumonia, whooping cough and some other infectious diseases. There are toxemias, moreover, notably lead poisoning, which carry in their train infectious diseases clinically and pathologically of an encephalitic nature and which deserve consideration in this connection.

Of all the conditions enumerated, the one which has aroused the most discussion during the last few years is that of vaccination, not only because it is of great importance in regard to epidemic encephalitis, but because the occurrence of encephalitis from this cause might require a change in attitude toward the practice of vaccination.

It appears that as early as 1912, Turnbull and McIntosh observed postvaccinal encephalitis. This was before the epidemic of encephalitis, and these observations were published only when these investigators made their further studies on similar cases in 1926. The subject was not considered in its present connection until Luksch,[29] in 1924, described three fatal cases of apparently typical encephalitis in which the patients were taken ill ten days after vaccination. Since that time similar cases have occurred in Holland, Austria, France, England, Greece and other parts of Europe, the most thoroughly studied ones being those reported by Bastiaanse,[30] occurring in Holland between January, 1924, and June, 1925. There were thirty-four cases of this type which appeared during a period following from nine to fifteen days after vaccination, although there was no violent reaction at the site of vaccination. The attacks usually came on rather suddenly with elevation in temperature; the patients developed somnolence, sometimes convulsions and a variety of symptoms often involving the cranial nerves. Fourteen of the thirty-four patients died. Bastiaanse found certain differences in distribution of the lesions in these cases from those in typical epidemic encephalitis, but the nature of the individual lesions was similar in the two diseases. There were, of course, during this time, enormous numbers of vaccinated children, and relatively few developed the encephalitic sequelae. In his analysis, in large towns of over 100,000 inhabitants, there was only one case to every 30,000 of the population, and one in every 9,000 vaccinations. In towns with less than 5,000 inhabitants, there was one in every 2,000 of the population, but one in every sixty-three vaccinations. A necessarily inaccurate, but nevertheless important, compilation, including Holland and Switzerland, estimated from 1 to 1.4 cases of encephalitis to about 10,000 vaccinations. Figures which I have taken from data compiled by the Committe of the Ministry of Health of Great Britain [31]

29. Luksch: Med. Klin. **8**:1170, 1924.

30. Bastiaanse, F. S.: Bull. Acad. de méd., Paris **94**:815, 1925; Nederl. Tijdschr. v. Geneesk. **2**:1267, 1926.

31. Lancet **1**:617, 1927.

show that there were about sixty-two cases of this kind in England from 1922 to 1924; thirty-six in Holland and ten others scattered in various parts of the continent. Winnicott and Gibbs [32] report other cases in 1926.

A comparison of the pathologic changes in postvaccinal encephalitis and in epidemic encephalitis has been considered in a previous section, and it may be accepted that the two conditions are not completely identical. On the other hand, the cases of Lucksch seem to have shown little difference from the encephalitic type, and those of Bastiaanse were different chiefly in localization, and even Turnbull and McIntosh admit considerable similarity between the histologic pictures of the two diseases.

The first thought that arises in reading these reports is the one that cutaneous vaccinations have been followed, in certain predisposed persons, by the entrance of an especially neurotropic vaccine virus into the brain; and this thought is rendered logical by Levaditi's [33] success in adapting a vaccine virus to invasion of the central nervous system. Also, Turnbull and McIntosh, in two cases of postvaccination encephalitis, determined the presence of vaccine virus in the brain and spinal fluid of the patients. These, however, are the only recorded instances of positive observations. I have failed in a similar case, and other observers have not been able to confirm these observations. It is more than likely that the success of Turnbull and McIntosh merely demonstrated a fact well known that within two weeks or so after vaccination vaccine virus is apt to be present in many of the organs of the body.

The problem has received a great deal of attention because of its obvious significance. The British Commission, the report of which is thoroughly analyzed in the *Lancet* of March 19, 1927, after a consideration of different views, summarize their opinion as follows:

1. Enormous numbers of children have been vaccinated in the past without resulting encephalitis, and a large number of inocuous contemporary vaccinations are still being carried out.

2. Except in a few cases, vaccinia virus has not been found in the brains of patients with fatal cases, and even if found, it would not have a necessarily specific consequence.

3. Large numbers of children have been vaccinated without harm with the same strains used for those who subsequently developed encephalitis.

4. The distribution of the cases of postvaccinal encephalitis has shown strong evidence of a grouping of cases at a certain time in certain places, which seem to determine the incidence.

5. The histologic differentiation between the postvaccinal cases and the epidemic ones described by Turnbull and McIntosh are not universally accepted.

6. The clinical features of the postvaccinal cases suggest the epidemic disease.

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32. Winnicott, D. W., and Gibbs, N.: Brit. J. Child. Dis. **23**:107, 1926.

33. Levaditi, C.: L'étiologie de l'encéphalopathie post-vaccinale, Presse méd. **35**:161, 1927.

I have not quoted these points in the exact language of the Commission, for purposes of abbreviation. To conclude, chiefly from this reasoning: it is definite that vaccination is at least a contributory cause, but that the immediate cause is probably some distinct agent.

Levaditi,[33] who has done a great deal of experimental work on the neurotropism of vaccinia virus, adds the important observation that from over 20,000 vaccinations in Spain with his neurovaccine, which is a vaccinia virus adapted to the central nervous system of rabbits, not a single case of encephalitis has resulted. Moreover, he has shown that his neurovaccine is practically nonvirulent for the lower order of monkeys when injected intracerebrally directly from the rabbit. Levaditi admits that absolute certainty is impossible at the present time, but he believes that a consideration of all the clinical and experimental evidence tends to indicate that the Jenerian vaccine is not the direct cause of postvaccinal encephalitis. His view appears to be the most rational that clinical observation and experimental evidence justify at the present time, and seems to me especially logical in view of the concentration of cases of postvaccinal encephalitis in localities and groups, at a time when large numbers of similar vaccinations with the identical virus are being made elsewhere without encephalitic sequelae. This indicates with much probability that the view is correct which holds that the vaccination, with its probable distribution of vaccine virus through the body, constitutes a predisposing influence which permits the invasion of an independent agent either present in the vaccinated person or freely distributed in the group at the time at which the vaccinations are carried out.

The observations made in cases of epidemic encephalitis and the peculiar difficulties of understanding the condition, especially in regard to its relation to the postvaccinal variety, impelled physicians to include in their considerations various similar conditions of the nervous system which have long been known to follow in the train of other infections and toxemias. It is, for instance, well known that encephalitis occasionally follows measles. Rolleston[34] states that encephalitis follows measles more frequently than it does any of the other exanthems. In 1927, Neal and Applebaum[35] published the report of twelve cases of clinically typical encephalitis not differentiable from the epidemic variety, which followed a few days or even two weeks after the onset of the measles and had a mortality of 25 per cent. Four cases, for which I am indebted to Dr. McKhann, occurred at the Boston Children's Hospital between 1923 and 1925, and the reports of many other cases have

34. Rolleston, J. A.: Acute Infectious Diseases, New York, Physicians and Surgeons Book Company, 1925, pp. 25, 33, 93.

35. Neal, J. B., and Applebaum, E.: Encephalitis Associated with Measles, J. A. M. A. **88**:1552 (May 4) 1927.

appeared in the literature. Speaking of them, Winnicott and Gibbs state that "there may be no definite acute encephalitis, but only the gradual onset of sequelae, exactly as one finds quite commonly in an epidemic of encephalitis lethargica."

Within the last few months, Musser and Hauser [36] have reported eight cases of encephalitis following measles which occurred during an epidemic of measles in New Orleans. The pathologic processes in two of these cases were studied, and the lesions described and illustrated by them are in their individual characteristics closely similar to those in epidemic encephalitis. There were many punctate hemorrhages; there was an accumulation of red cells in perivascular spaces; there was perivascular cuffing with lymphocytes, and the description might well be that of an epidemic case—at least, in my opinion. The hemorrhages were particularly concentrated in the basal ganglions.

Varicella encephalitis is not uncommon and the literature on this condition has been carefully reviewed by Winnicott and Gibbs. They cite thirteen such cases from other investigators, adding two of their own. They believe that these cases differ somewhat in clinical signs from the encephalitic type. They state, nevertheless, that although varicella is a common disease, only a few cases in which there were nervous complications have been reported. The relationship in time between the onset of the varicella and the development of encephalitis leaves little room for questioning the direct relationship between the two conditions, but it is interesting that these authors add that there is not any obvious correlation between the severity of the attack of varicella and that of encephalitis. At the time of their writing, June 26, autopsies on such cases were not available.

In a review in the *Sweizer Medizinische Wochenschrift*,[37] in addition to five further cases of varicella encephalitis, thirteen cases are reported following smallpox which apparently were similar to the postvaccinal cases.

The great variety of preliminary diseases which has led to encephalitic processes may be well illustrated by a list of clinically definite cases of encephalitis following various conditions, as observed between 1923 and 1925 at the Boston Children's Hospital, and kindly placed at my disposal by Dr. McKhann of the staff of that hospital.

His cases, the report of which will be published with detailed comment within a short time, are classified as follows:

Abscess of a Tooth: One case, typical, following immediately on involvement of tooth.

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36. Musser, J. H., and Hauser, G. H.: Encephalitis as a Complication of Measles, J. A. M. A. **90**:1267 (April 21) 1928.

37. Review in Schweiz. med. Wchnschr., Feb. 12, 1927.

German Measles: One case; definite, following immediately on rash.

Measles: Four cases, within two weeks of rash.

Lead Poisoning: Sixteen cases, coming on with a sudden onset a few months after the beginning of the lead poisoning. In one of these cases, autopsy showed a typical encephalitic histology.

Pneumonia: One case, developing during convalescence.

Bronchitis and Involvement of the Upper Respiratory Tract: Seven cases, usually coming on within five days after disease.

Vaccination: Two cases; in one of these this laboratory failed to demonstrate vaccinia virus in the nervous system.

Acute Enteritis with Vomiting and Diarrhea: Two cases, developing shortly after disease.

Whooping Cough: One case; similarly definitely related to the primary condition.

The important question immediately arises whether such an obviously secondary development of encephalitis, from whatever cause, can justly be classified with epidemic encephalitis on an etiologic basis. This is particularly in doubt in such cases as those following lead poisoning, in which a definite and independent toxic agent is found in the nervous system at the time when the disease occurs, and these cases are included only because they show that a simple toxemia by a known agent can cause a disease clinically similar to the epidemic variety. In the pathologic changes, moreover, these cases of lead poisoning show much similarity, in regard to hemorrhage and round cell infiltration, to the epidemic disease, and in a general review of the subject they should not be omitted.

The interpretation obviously can be two-fold: either these various developments in encephalitis may be regarded as a common result of many different toxic agents, or they can be interpreted in the sense in which Levaditi regards them; namely, that they all represent the preparation of the soil in a susceptible person by a noxious agent for the invasion of a specific virus. In this connection, it is again worth noting that the cases occurring at the Children's Hospital in Boston, while scattered over two years, were in considerable numbers concentrated during two definite periods in which six or seven came together within the short time of two weeks.

The question is one that cannot be answered, as is the case with so many other problems in this disease, but to present all the material for reasoning available at the present time, it is necessary to pose it. And it is important to notice that while the so-called predisposing causes include many entirely separate insults, most of them—varicella, vaccination, smallpox and mumps—are probably agents belonging to the so-called filtrable virus group, and influenza—the most important of all—is suspected of belonging to the same class.

It is also interesting to note that Bassoe, in discussing eleven cases of typical encephalitis observed by him, could not find any definite disease preceding the neurologic condition, but was so impressed with the exhausted and debilitated condition of his patients prior to their attacks, that this alone suggested to him that the disease was due to a separate virus that had probably become neurotropic only because of the predisposition of his patients.

THE ETIOLOGIC PROBLEM

Bacterial Causation.—In encephalitis, as in most other diseases of obscure etiology, a number of different bacteria have been held responsible by various observers. From the early cases described by von Economo in Vienna, von Wiesner [38] isolated a diplostreptococcus which was seriously discussed for some time. Von Wiesner inoculated a monkey subdurally with material from the brain of a person who had had encephalitis. The monkey died in two days, and the organism was cultivated from this animal. Subsequent inoculations into monkeys and rabbits proved the organism highly virulent for both species. Apart from any of the other objections to a bacterial etiology which will be discussed subsequently, the results of von Wiesner can be ruled out purely from the effect this organism had in animals. The ease with which both monkeys and rabbits succumbed is out of keeping with the large number of subsequent attempts at inoculation of animals of these species.

Rosenow [39] has also cultivated streptococci from cases of encephalitis, and Maggiora [40] and his associates, as well as Ottolenghi and Tonietti,[41] have obtained similar gram-positive cocci.

The latest report of the cultivation of a streptococcus-like organism from cases of encephalitis is that of Evans and Freeman,[42] which I have found necessary to take seriously because of the known accuracy of these investigators. Not only did they isolate a streptococcus from the material of encephalitic cases by a special meat broth technic, but they have found the bacteria histologically in the brain, and they believe that in the brains of encephalitic patients most of the organisms may assume a filtrable form. Filtrates of emulsions in the brain of human beings did not cause disease in inoculated rabbits, but filtrates planted in meat medium resulted in streptococcus cultures, and from this Evans and Freeman inferred "that the organism is in a filtrable form in the human brain and in the rabbit brain and in cultures."

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38. Von Wiesner: Wien klin. Wchnschr. **30**:933, 1917.
39. Rosenow, E. C.: J. Infect. Dis. **34**:329, 1924.
40. Maggiora, Mantovani and Tombolato: Riforma med. **36**:114, 1920.
41. Ottolenghi, D.; d'Antona, S., and Tonietti, F.: Policlinico **27**:1075, 1920.
42. Evans, A. C., and Freeman, W.: Pub. Health Rep. **41**:1095, 1926.

The problem in regard to the work of Evans and Freeman is of much the same order as that regarding the streptococci claimed by Rosenow in poliomyelitis; and a strain of virus placed at my disposal by Dr. Evans has been found, in many passages in rabbits to be identical pathogenically and immunologically with strains of herpes virus. A large number of cultivations made by my associates, Drs. Tang and Yu, from rabbits infected with this strain have occasionally yielded streptococci, but the organisms so obtained have repeatedly failed to produce a disease in other rabbits anything like that produced by the brain substance itself. If one assumes the possibility of a mutation of an organism into a filtrable virus form, and together with that, a modification in pathogenic properties during the course of this mutation, one could accept as a solution of the problem not only the work of Evans and Freeman, but that of others who have cultivated bacteria. It is still a far cry experimentally to such a premise, however, and logic forces one at the present time to reject the bacterial causation of this disease on the following grounds:

1. The clinical symptoms and speed of death of inoculated animals not only is different from that of the human disease, but differs materially from that following the inoculation of the herpes virus into the same species of animals.

2. The pathologic changes in encephalitis in man are fundamentally different from those produced by any of the known forms of bacteria.

3. The nature of immunity, as far as is known in encephalitis, and certainly in filtrable virus diseases generally, and herpes especially, is different, both biologically and serologically, from that familiar in streptococcic infections.

4. In addition to this, all those who have seriously claimed bacterial etiology for this disease have assumed an interrelationship between the bacteria and a filtrable stage, in which case it would be necessary to stretch this mutation not only into one of form and infectious properties, but into one affecting all the basic biologic attributes of the agent; for—unlike bacteria—the filtrable virus can be preserved in glycerin indefinitely, degenerates easily in salt solution, and is much less resistant to heat, chemicals and most other deleterious agencies than are bacteria.

Altogether, then, a poor case is made out for bacterial causation, which must be thrown out entirely, except on the slim possibility of actual mutation, for which the evidence is entirely too unreliable at the present time to permit anything more than a purely speculative view.

For the reasons previously mentioned, therefore, there are few investigators who still favor a bacterial etiology for epidemic encephalitis and although a continuation of such studies is perhaps desirable, both in this disease and in poliomyelitis, in order that this difficult field may be completely covered, most workers have been led to the tentative assumption that the agent which causes encephalitis belongs to the group of the so-called filtrable virus. Such a point of view is eminently logical, since poliomyelitis and rabies, both of them diseases of the central

nervous system, and both of them displaying pathologic changes which show much similarity to those of encephalitis, are caused in all probability by filtrable agents.

The etiologic problem as it stands at this point, therefore, is as follows: Physicians are confronted with a disease of the nervous system which, in its epidemic occurrence and in its evidence of transmission, must be regarded as an infectious disease. In the relative infrequency of direct evidence of infection from one case to another, and in distribution, it possesses close analogy to poliomyelitis and to rabies; at least, the basic cellular responses of the nervous tissues are sufficiently alike in these conditions to point to the involvement in encephalitis of an agent belonging to the same general group as those causing the disease mentioned.

When, therefore, during the course of investigating the facts of herpes virus in man, Doerr and Voechting [43] discovered that this agent could become neurotropic and give rise to encephalitis in rabbits, and when herpes virus was isolated from the spinal fluids and brains of several patients with encephalitis, it was natural that an association between herpes and encephalitis should be considered.

In order to discuss this phase of the problem as comprehensively as it deserves, I shall need to consider the facts which have been discovered regarding herpes as a separate disease and the virus which causes it.

Encephalitis and the Virus of Herpes.—Long before there was any knowledge, through Grüter's work,[44] of the transmissible virus contained in herpes vesicles, there was a tendency to regard herpes as an independent clinical entity which, though usually an accompaniment of other conditions, could appear by itself and give rise to systemic symptoms in addition to the local lesion. Achard [45] summed up this point of view in 1925 by saying that there were cases in which the appearance of herpetic vesicles was accompanied by fever, general pains and headache, in which no other cause for these systemic manifestations could be detected. I have seen one such case within the last two years, in which a vague grip-like syndrome, appearing periodically, was followed in a day or so by a herpetic eruption behind one ear.

Another point pertinent to this problem is the suspected association of the herpetic eruption with injuries, by either disease or operative intervention, of the peripheral nervous system. Levaditi cites cases reported by Achard and Laubry,[46] Clement, Simon and Pauthier [47] and

43. Doerr and Voechting: Rev. gén. d'opht. **34**:409, 1920.

44. Grüter: Arch. f. Augenh. **70**:241, 359, 1912.

45. Achard: Zona et herpes, Paris, J. B. Bailliere et fils. 1925. p. 65.

46. Achard and Laubry: Gaz. hebd. de méd. **28**:1129, 1901.

47. Clement, Simon and Pauthier: Bull. et mém. Soc. méd. d. hôp. de Paris, 1907, p. 1295.

others in which herpes followed the injection of cocaine into the spinal canal. Gerhard [48] believed that facial herpes was related in some manner to lesions of the trigeminal nerve, and Cushing [49] has made similar observations in connection with operations on the gasserian ganglion, herpes appearing only when the ganglion is preserved and rarely when it is removed. Indeed, Ravaut [50] gives this association the name "Herpes Neuralgique." This relation is particularly noteworthy, of course, in its bearing on the present problem, in which encephalitis is regarded by many observers as due to an increased development of neurotropism on the part of the herpetic virus. Another suggestive fact is the frequency with which recurrent herpes of the skin seems to reappear again and again in the same spot in certain persons after intervals of many months. Levaditi cites a number of cases of this kind; others have been reported by Poincloux,[51] Flandin and Tsanck,[52] and I have seen several—one in particular, in which, at yearly intervals, a young man developed typical, and transmissible herpes over the dorsum of the left thumb. Such local recurrence necessitates the assumption of the latency of the virus in the tissues, and the peculiarities of distribution suggest the terminal nerve fibers as the reservoir of survival. Most important to remember is that, like encephalitis itself, herpes is most often secondary to other injuries by which the body is in some manner rendered susceptible. The implications of the words "cold sore" are obvious, and other conditions, in which the appearance of herpes of the lips has attained an almost diagnostic importance, are meningitis, pneumonia, influenza, malaria and a number of other infectious diseases.

Since the inoculability of the herpetic virus from all these sources to rabbits is now a fact, and since it is certain that the various strains of the virus, whatever the source of the original human vesicle, can be shown to be identical by cross-immunization, it can only be assumed that, in its origin in man, the separate disease herpes is due to the activation of an agent latently present in the patient's body, and rendered capable of inciting lesions by circumstances prevailing in the individual case, pathologic change or operative injury of the nerves, or the toxemia of incidental diseases such as those mentioned.[53]

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48. Gerhard, cited from Levaditi: L'herpès et le zona, Paris, Masson & Cie, 1926, p. 21.

49. Cushing, H.: Am. J. M. Sc. **127**:375, 1904.

50. Ravaut: Nouveaux traites de médicine, second pamphlet, Paris, Roger Wiedal Taisier, 1921, p. 315.

51. Poincloux: Ann. de L'inst. Pasteur **38**:977, 1924.

52. Flandin and Tsanck, cited from Levaditi: L'herpès et le zona, Paris, Masson & Cie, 1926, p. 18.

53. Fischer, M.: Ztschr. f. Hyg. u. Infectionskrankh. **107**:102, 1927.

This point of view is further strengthened by observations in which herpes has appeared in the train of a variety of procedures in which extraneous infection with herpes virus can be excluded. A few years ago, during the course of typhoid vaccination, one of my colleagues suffered from a severe reaction after the second injection—vomiting, general pains and a fever which rose to 104 C. and which lasted over twenty-four hours. Immediately after this he developed herpes which was transmitted to rabbits and carried on for several passages. Fischer,[53] who has recently summarized similar observations, cites instances in which herpes followed therapeutic infections with malarial parasites, bacterial vaccine containing staphylococci, pneumococci, streptococci, meningococci, milk, colloidal metals and other substances. It is obvious from the entirely unrelated nature of the provocative materials and the demonstrable fact of a separate and uniform herpetic agent, that one must assume the presence of a chronic latency of the latter, whatever it may be, in the body of the subject. And since it is now certain that the inciting substance of herpes, though occasionally found in the saliva and in other parts of the body of man, is not often present in the normal secretions, one must assume what Doerr [54] and his school have called an endogenous ubiquity—namely, the frequent latent presence of the causative agent in the tissues of man.

The idea that herpes may be caused by a living infectious agent resulted in 1913 from the work of Grüter, who inoculated from vesicles on the cornea of man to the cornea of rabbits. Six years later, Löwenstein [55] repeated this work, and found that the experiment of Grüter was successful with considerable regularity, and that the virus could be passed from rabbit to rabbit. Since the time of Löwenstein's experiments, many other workers have repeated and confirmed his observations, and it is now well recognized that such transmission can be carried out with material from any human herpes—wherever it is localized—and at any stage of vesicle formation. I always except herpes zoster, which is a separate problem and which cannot be adequately treated in a brief manner. The association of the herpetic virus with encephalitis was not suggested, however, until Doerr and Voechting,[43] in 1920, noticed that rabbits suffering from a herpetic keratitis sometimes developed general symptoms which pointed to involvement of the nervous system. This led to a search for the virus in the nervous system of the animals, and it was found that encephalitis in rabbits could be passed in unlimited series by intracerebral inoculations of brain suspensions and filtrates, giving rise to a symptom-complex which is extraordinarily

54. Doerr: Centralbl. f. Bakteriol., 1, Orig., 1925, vol. 97, suppl.

55. Löwenstein: Klin. Wchnschr., 1919, p. 1222; Klin. Monatsbl. f. Augenh. **64**:15, 1920.

regular and which forms a uniform clinical entity. Individual strains of the herpes virus obtained from man vary considerably in the degree of their original neurotropism, but once implanted on the nervous system, though varying in virulence, they can be carried from brain to brain without the slightest difficulty. This agent bears considerable similarity to that causing poliomyelitis, in that it is filtrable and can be preserved in glycerin almost indefinitely; one of my strains was kept alive for twelve months in the icebox in 50 per cent neutral glycerin.

Herpes virus has been extensively studied and has been found analogous to other so-called filtrable agents. Like all the others, it has remained uncultivated, and nothing has been seen under the microscope which can be regarded as the agent itself. Ultrafiltration experiments carried on in the laboratory [56] of Harvard University Medical school have indicated its size as ranging between 20 and 100 millimicrons; this agrees in general with the measurements of the virus of foot and mouth disease obtained by Olitsky and Boez,[57] and is within the same range, according to my own measurements as the magnitudes of bacteriophage and the Rous sarcoma virus, and considerably larger than trypsin, even in an impure form. Of the greatest importance is the fact that in herpetic lesions, a number of observers have found the nuclear inclusion bodies which have been described by Lipschuetz in so many of the reactions caused by filtrable viruses. It is, as I have seen, pathogenic for rabbits, and will also infect guinea-pigs and, to a less extent, rats, and with still less regularity, certain species of monkeys.

The immunologic conditions, again, are analogous to those observed with other filtrable agents. Broadly speaking, without going into minor details, an animal that has recovered from a corneal inoculation becomes —within three or four weeks—immune to intracerebral inoculation, and remains refractory for at least four or five months. Active immunization can also be accomplished by the skin and by intracerebral inoculation with sublethal doses. It is important to the analogy with other filtrable virus diseases that there is no immunization unless a certain amount of reaction to the living agent occurs, dead virus lending no immunity; and living virus, completely neutralized with immune serum, is similarly ineffective. Animals that have been immunized possess substances in their circulating blood which neutralize, but such neutralization is effective only when serum and virus are incubated before injection, and not when serum is injected (even into the cisterna) into an animal already infected.[58] My recent experiments indicate that in

56. Zinsser, H., and Tang, F.: J. Exper. Med. **46**:357, 1927.
57. Olitsky and Boez: J. Exper. Med. **45**:673, 685, 1927.
58. Zinsser, H., and Tang, F.: J. Exper. Med. **44**:21, 1926.

such work past irregularities can be explained by the rapidity with which the virus deteriorates in salt solution at body temperature, and this deterioration has been shown to be largely due to oxidation.

It is natural that the discovery of the neurotropism of herpes virus should have led investigators to the association of this virus with epidemic encephalitis. Analogy with poliomyelitis had, of course, suggested inoculation of various animals with material from the central nervous systems of encephalitic cases. This method appeared to yield astonishingly regular results in the early reports of Loewe, Hirshfeld and Strauss of New York,[59] but the ease with which these results were obtained makes it apparent—in the light of later investigation—that there was some accidental error in their work. They possibly encountered a strain of rabbits suffering from the spontaneous encephalitis of rabbits described by Wright and Craighead[60] and studied by Bull,[61] Oliver,[62] Cowdry and Nicholson[63] and others. Likewise, one is at a loss to know what to say about the report of the New York workers that they cultivated the virus and transmitted the disease by means of the cultures. McIntosh and Turnbull[27] also published a report of the positive inoculation of a monkey (*Cercopithecus*), a fact that should be noted for further reference, though it is not clear what this unquestionably reliable experiment means. They were not capable of transmitting from this monkey. Many similar attempts have been made all over the world, and it has been a matter of extraordinary difficulty to differentiate between false leads and significant facts. The Koritschoner virus was probably a rabic virus isolated from an erroneously diagnosed case,[64] and the virus of Kobayashi, obtained from the epidemic in Japan in 1924, has been shown by Cowdry[65] unquestionably to be rabies. I have no definite information concerning the Takagi virus from the same epidemic. Kling and Liljenquist, in all probability, were misled by spontaneous encephalitis of rabbits; this is the opinion of as experienced an observer as Levaditi.

When everything is eliminated that seems obviously to be irrelevant, there remain only a few instances in which strains of virus of any

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59. Loewe, L.; Hirshfeld and Strauss: New York M. J. **109**:772, 1919; J. Infect. Dis. **25**:378, 1919; Etiology of Epidemic Encephalitis, J. A. M. A. **73**: 1056 (Oct. 4) 1919.

60. Wright and Craighead: J. Exper. Med. **36**:135, 1922.

61. Bull, C. G.: J. Exper. Med. **25**:557, 1917.

62. Oliver, J.: J. Infect. Dis. **30**:91, 1922.

63. Cowdry, E. V., and Nicholson, F. M.: Meningo-Encephalitic Lesions and Protozoan-Like Parasites in Brains of Apparently Normal Animals Commonly Employed for Experimentation, J. A. M. A. **82**:545 (Feb. 16) 1924.

64. Doerr and Zdansky: Ztschr. f. Hyg. u. Infectionskrankh. **102**:1, 1924.

65. Cowdry, E. V.: J. Exper. Med. **45**:799, 1927.

kind were definitely isolated from the central nervous system of encephalitic cases, and these can be briefly enumerated as follows:[66]

1. A strain obtained by Levaditi and Harvier by the inoculation of brain material into rabbits in 1920.

2. A strain obtained by Doerr and Schnabel from spinal fluid.

3. A strain obtained by Doerr and Berger by inoculation of brain material in 1922.

4. The strain of Berger, obtained by inoculation of brain material. In this case there was an interesting phenomenon, to be discussed later in connection with Perdrau's cases—namely, that the fresh material did not give any results, but the inoculation of the same material glycerinated for two weeks produced typical herpes encephalitis in rabbits.

5. The strain of Schnabel, obtained from spinal fluid.

6. The strain of Doerr and Zdansky, obtained in 1924 from glycerinated material of the brain sent them by Kling.

7. The strain of Luger and Lauda obtained from spinal fluid.[67]

8. The several strains of Perdrau[68] in England, which will be considered in greater detail subsequently.

The chain of evidence which seems to lead to the association of herpes virus with Economo's disease may, therefore, be summarized as follows: Herpes virus is frequently present in the body of man, where it causes—either spontaneously or, more often, secondary to some provocative condition—characteristic lesions on the skin, the mucous membranes or cornea; experiment has shown that the herpetic virus may develop neurotropism and cause encephalitis in animals; the virus has been recovered from the brain and spinal fluid in fatal cases of epidemic encephalitis in man.

These facts would appear to compel the acceptance of an etiologic relationship, were it not for a number of important difficulties. Foremost among these is the occasional presence of herpes virus in the spinal fluid of persons who are not suffering from, nor have ever had, Economo's disease. Bastai and Busacca,[69] who have given this phase of the question much attention, believe that in all cases of extensive cutaneous herpes the agent penetrates the body generally. They claim to have shown its presence in spinal fluid and blood in about 68 per cent of persons examined by them. Observations of such obvious importance have necessitated extensive reinvestigation, which has

66. In his book, L'herpés et le zona, published in 1926, Levaditi lists all these strains except those obtained by Perdrau in England, which I consider particularly important, for reasons to be given subsequently.

67. Luger and Lauda: Wien. klin. Wchnschr. **21**:386, 1923.

68. Perdrau: Brit. J. Exper. Path. **6**:41, 1925.

69. Bastai, P., and Busacca, A.: Acad. di Medicine di Torino, 1923 and 1924; Klin. Wchnschr. **3**:147, 1924; München. med. Wchnschr. **71**:1056, 1924; Schweiz. Arch. f. Neurol. u. Psychiat., 1924, vol. 15, no. 2; 1925, vol. 16, no. 1.

generally yielded negative results. Fischer,[53] of Doerr's laboratory, failed to confirm the observations of Bastai and Busacca in any of forty-three examinations. Although, therefore, the far-reaching generalizations of the Italian observers are surely not justified, the actual presence of the virus without encephalitis is proved beyond question in a case reported by Flexner.[4] Flexner obtained a typical herpetic virus by the inoculation of two rabbits with the spinal fluid of a tertiary syphilitic patient. Observations such as these, though equally rare, detract considerably from the conclusiveness of the occasional discovery of an herpetic virus in encephalitic patients, and, of course, a much larger number of inoculations of such pathologic materials has been carried out than of similar substances from normal persons or in other types of disease. They lend logic to the opinions, expressed particularly by Parker and by Flexner, that, after all, the herpetic virus discovered in the brains in a few encephalitic cases might be accidental admixtures having no relationship to the disease.

On the other hand, neither the occasional presence of herpetic virus in the nervous systems of normal persons nor the failure of intravenous injection of herpes to cause encephalitis in normal people[70] constitutes conclusive refutation of the suggested etiologic association. For, even without my own serologic determinations of the immune state of many normal adults, the nature of the epidemiology of encephalitis would lead me to assume such a condition; and, by analogy with many other diseases—meningitis, typhoid fever, diphtheria and others—I know that in any community there is always a greater number of carriers than of active cases of the respective disease. The objections to the etiologic rôle of the herpes virus on these grounds, therefore, while they must be given due consideration, are not negatively conclusive.

Another and as significant an obstacle to the herpes theory is the infrequency with which this virus has been recovered from cases in human beings, in spite of a large number of attempts made by experienced observers to find this virus. As far as I know, in more than one hundred attempts, Flexner and Amoss have not had any success, and this has been my experience in a large series of inoculations from at least eight reliably diagnosed cases. And these are only the American examples of an almost universal experience. It is, of course, difficult to harmonize such rare observations with an etiologic relationship, but there are many considerations which should dissuade one from too hasty rejection of the possible association because of this irregularity. It is well known that many filtrable agents may lose viru-

70. Grüter: München. med. Wchnschr. **31**:1058, 1924.

lence for one species of animals as they pass through another, and a neurotropic herpetic virus which has been in the nervous system of man for a long time may well have lost its virulence for the rabbit. The converse is well known with virus fixé of rabies, which becomes relatively avirulent for man by passage through rabbits. Dimitrieff,[71] indeed, claims to have shown that herpes virus passed through the brains of guinea-pigs loses in virulence for rabbits. Again, the majority of encephalitic cases which have come to autopsy since this method of investigation has been common have usually been in the chronic stages, and the virus may be not only attenuated, but considerably diluted. There are indications, moreover, that a herpes virus may actually be present in human material, so altered that only by special methods can positive rabbit inoculation be accomplished. The evidences for this are the experiences of Berger,[72] of Perdrau and of McIntosh and Turnbull. The first two investigators, inoculating rabbits with encephalitic brain tissue of man, obtained negative results when the material was fresh. After the brain tissue had been preserved in glycerin for two or more weeks, however, and then transferred to rabbits—in Perdrau's cases by special methods—the rabbits developed typical herpes encephalitis. Perdrau's virus is now in this laboratory and is identical in every respect with ordinary herpes strains. McIntosh and Turnbull [27] obtained a positive result in a monkey with similarly glycerinated materials, but—and this seems particularly significant—they were unable to transfer from this animal to other monkeys. It is true that I have utterly failed to confirm Perdrau's work. It must be remembered, however, that with so many variants in regard to the nature of the original material used, negative results do not utterly invalidate the positive observations of honest investigators, especially when several observations of the same phenomenon carried out in different laboratories are available. A corollary to the McIntosh-Turnbull observation, furthermore, is one reported by Levaditi.[6] This worker, admitting the usual resistance of monkeys to this virus, obtained a positive result in a *Macacus cynomolgus* inoculated with a strain of virus that had been passed through rabbits. This animal—one of three—developed a pathologically typical encephalitis, but material from this monkey—as in the McIntosh-Turnbull case—did not infect rabbits. It should also be remembered, in appraising these isolated, apparently accidental, but significant observations, that most of the successful transmissions from man were made from cases much more acute than those that have been available in this or other countries during the last four or five years.

71. Dimitrieff: Ztschr. f. Hyg. u. Infectionskrankh. **106**:547, 1926.
72. Berger: Wien. klin. Wchnschr. **41**:801, 1922.

Positive results in monkeys inoculated directly either with material from man or with strains carried on rabbits have been relatively rare, of course; but in those that have been obtained, both the clinical course and the pathologic histology have been definitely more comparable to the human disease than to that of the rabbit. It may well be that the considerable differences between herpes encephalitis in rabbits and conditions in man and monkey may be due in part to the rapidity with which the rabbit succumbs.

Immunology has helped little in solving this problem. A great deal has been done to elucidate the conditions of herpes immunization, but this has so far contributed little to the determination of its relation to encephalitis. My own unpublished measurements of the viricidal properties of convalescent encephalitic serums for herpes virus point in the direction of such a relationship, but the series is still too small to justify argument.

With the facts already outlined, then, the problem has reached an impasse in which it must remain until further light is available. Positive opinion is always an impediment to progress if evidence is obviously insufficient to justify it. I cannot join Levaditi in his expressed conviction that the herpes virus in a somewhat altered condition, represents the actual virus of epidemic encephalitis. And I must agree with Flexner in his statement that none of the virus strains so far isolated can be conclusively accepted as the causative agents of Economo's disease. On the other hand, I am strongly inclined to the opinion that the herpes virus and that of encephalitis are either identical in the sense of Levaditi, or closely related, for the following reasons—in which I may summarize the material presented in this paper.

Epidemic encephalitis is beyond doubt an infectious disease in which the virus causes lesions in the central nervous system, comparable in histologic principles to those in poliomyelitis and to some extent, to those in rabies. While these lesions might be interpreted as purely toxic, the living agent being localized elsewhere in the body, the aforementioned analogy renders this unlikely. The epidemiology, course and pathologic changes in epidemic encephalitis render it probable that one is dealing with a disease caused, not by bacteria, but by an agent of the so-called filtrable virus group which invades the central nervous system.

There is considerable analogy between herpes and encephalitis as secondary diseases. Even though one admits that the many varieties of encephalitis following grip, measles, mumps, whooping cough, vaccination, varicella, smallpox, and other infectious diseases have not been proved to be identical with the so-called primary epidemic encephalitis, yet, throughout the study of the disease, clinicians have again and again been impressed with the fact that many of their cases have been

preceded by some other malady or injury, so much so that theories of such a relationship have repeatedly been forced into the foreground. In herpes, the secondary nature of the disease is even more conspicuous.

Herpes virus must be regarded as frequently present in the tissues of man, usually giving rise to cutaneous, mucous or corneal lesions. Its neurotropism having been shown in animals, and its presence in encephalitic patients having been rarely but definitely demonstrated, it is assumed that herpes virus, usually localizing in the integuments, may become neurotropic in persons specially predisposed by debilitation or preceding provocative illness.

The infrequency with which it has been demonstrated in cases is tentatively explained by considerable modification for animals by its prolonged presence in the nervous system of man and by great attenuation during the course of prolonged chronic disease. It is pointed out that most successful transmissions from man occurred during the earlier phases of the epidemic, when more acute cases came under observation. The occasional presence of the virus in nonencephalitic persons is easily comprehended on the basis of a sort of carrier state in an immune subject, and it is more than likely that a large majority of human adults possess a partial or complete immunity as the result of repeated cutaneous lesions.

It is clear from all that has been said before that the relationship between the herpetic agent and encephalitis in man is an unsolved problem. Nevertheless, there is a great deal to be said in its favor, and it is an hypothesis that cannot wisely be left out of consideration in further seeking light on this disease. Incidentally, the pathologic and clinical nature of Economo's disease is such that there can hardly be any doubt about its being caused by an agent belonging to the same class of so-called filtrable viruses as do the causative agents of herpes, poliomyelitis and probably rabies, vaccinia and a number of other diseases. Moreover, in the herpes virus one is in the possession of a substance which can be utilized on a large scale for the production of experimental encephalitis in animals, and I believe that a clearing up of the various conditions governing the herpetic disease in rabbits is the most logical direction of investigation at the present time for the elucidation of the problem of encephalitis.

THE GENERAL NATURE OF THE SO-CALLED FILTRABLE VIRUSES

The discovery that many diseases are caused by agents that are too small to be held up in the pores of filters which obstruct the passage of even the smallest bacteria naturally led to the assumption that such diseases were caused by ultramicroscopic living cells. The new knowledge which has come largely through observations of the bacteriophage, how-

ever, has led to interesting speculations that are too important to omit in any contemporary discussion of filtrable agents. It is, of course, well known that there is an active controversy between two schools: one of these, that of d'Herelle, believes that the bacteriophage is an ultramicroscopic virus or living agent; the other, that the lytic action of the bacteriophage is analogous to that of autolysis, the process being initiated by the bacteriophage filtrate more or less in the manner in which a catalytic agent initiates many reactions. The phenomenon is a complicated one and cannot, of course, be dealt with exhaustively in passing, but it may be well to call to mind that the filtrate of a culture dissolved by bacteriophage acts only on living and actively growing cells under favorable cultural conditions, and that there are many indications that suggest that the growing bacteria which are being dissolved are directly responsible for the reproduction of the lytic principle. In other words, a crude description of the process would be as follows: Once a bacteriophage for a given culture has been started, usually in the intestine or in glycerinated vaccine pulp or under other conditions in which the bacteria are in contact with the autolyzing cells, something is produced which initiates lysis of similar bacteria; and these bacteria in turn, while undergoing lysis, reproduce the lytic principle. This principle is filtrable and in a great many important attributes is analogous to filtrable virus agents in general. Could one conceive a cytophage which would initiate a similar autolytic process in cells of the nervous system, not only would the injuries produced by such an agent account for the pathologic conditions and lesions, but they would explain experimental transmission from animal to animal, since such a cytophage would, in complete analogy with bacteriophage, be reproduced by the specific cells which degenerated because of its action. Even the specificity for tissues of the nervous system, for instance, would be in keeping with such an analogy, since it is known that in the case of bacteriophage, a given lytic agent may be specific for only one culture and a few of its closely related species, or may even be specific for a few races of the species only.

It was Twort, I believe, who first suggested that in some of these filtrable virus diseases one may be dealing with a cytophage material perhaps not cellularly organized. This idea, which has since occurred to many workers in this field and which has been particularly discussed by Doerr,[73] would be consistent with the fact that neither the bacteriophage nor any of these filtrable agents have ever been definitely cultivated. Doerr, who has given the subject a great deal of thought, cites early experiments of Iannovics, who was impressed with the occasional encephalitis-like consequences following months or longer after gunshot

73. Doerr: Nederl. Tijdschr. v. Hyg., Microb. en. Sero., November, 1926, vol. 3.

wounds of the skull. Iannovics [74] drew the conclusion that traumatic areas in the brain might lead to an absorption of dead tissue which secondarily acted on the surrounding traumatized areas. He experimentally produced similar trauma in rats by contusions of the skull and the injection of suspensions of the brain of rats, and claims that in these animals a disease developed not unlike the slow, encephalitis-like conditions following the injuries of the skull in man. He also suggests that the occasional nerve lesions reported in the course of antirabies inoculations, which at best are rare, may be explained in a similar manner as the result of treatment with nerve tissue.

This train of thought obviously reminds one of the apparent influences of various injuries of the body on the development of herpes and encephalitis in man, which—as has been seen—are often secondary to other conditions and, especially in herpes, follow not only the toxemia of infectious diseases, but such unrelated factors as the injection of dead bacteria or even lumbar puncture. Such a conception might also explain in an entirely new way the supposed development of encephalitis from the injection of bacteria into the brain, which has been interpreted by those who claim to have observed it as a mutation of the bacteria to a nltrable form.

Particularly pertinent to the subject are the experiments of Rivers and Tillett [75] in the production of what they speak of as their "Virus 3." Rivers and Tillett, in the course of attempts to transmit varicella to rabbits by intratesticular injection, observed moderate inflammatory reactions developing within a few days, and by successive transfer of testicular tissue from rabbit to rabbit obtained, after the fourth transfer, a reaction which could be transmitted in series with increasing strength. The reactions in the rabbits could be shown to be due to a filtrable agent, preservable in glycerin and having many of the other attributes of filtrable viruses, and was definitely shown not to have any relationship to varicella. Based on these observations of Rivers, Miller, Andrewes and Swift [76] studied similar reactions obtained in testicles of rabbits which, in the original animal, had been started by the injection of blood and joint fluids from patients suffering from rheumatic fever. Andrewes and Miller,[77] following out the idea of Rivers that perhaps the virus had been present in the rabbits used for his experiments on varicella and had merely been activated by the trauma of the testis, inoculated the blood of apparently normal rabbits into the testicles of rabbits. In two of

74. Iannovics, quoted from Doerr: Ztschr. f. Hyg. u. Infectionskrankh., vol. 112, p. 49.

75. Rivers and Tillett: J. Exper. Med. **38**:673, 1923; **39**:777, 1924; **40**:281, 1924; **43**:275, 1926; **45**:11, 1927.

76. Miller, Andrewes and Swift: J. Exper. Med. **40**:773, 1924.

77. Andrewes and Miller: J. Exper. Med. **40**:789, 1924.

six transmission series, they produced a similar transmissible virus, identical in every way with the Virus 3 of Rivers; they obtained indurated and clinically positive lesions after the fourth and fifth passage in a series.

Carrel's [78] work on the production of tumors in chicken is also of the greatest significance in this connection. It is particularly interesting because of the many analogies between the filtrable agent of the Rous sarcoma and herpes virus. Carrel states that the Rous substance dissolved in Tyrode solution disappears after an incubation of fifteen hours, while it is still active if mixed with serum. This is exactly analogous to my experience with herpes virus, a phenomenon which I have interpreted—following the experiments of Mueller with cysteine and the Rous substance—as meaning that these agents are rapidly inactivated by oxidation. Carrel has apparently been able to produce in chickens a series of tumors transmissible with filtrates and in every way analogous to the Rous tumor by injecting embryonic pulp at the same time with solutions of indol, arsenic and tar. By a combination of the irritation and the implantation of embryonic cells, he was able to initiate a sarcomatous degeneration of these cells which developed into growing tumors that yielded a virulent filtrate. These observations persuaded Carrel to conclude that the filtrable incitant of fowl sarcoma is not a living agent, and while, of course, other interpretations might be applied, his point of view is perfectly reasonable and seems the most simple explanation of the experimental facts.

There is, thus, a considerable amount of analogy between the bacteriophage phenomenon and the filtrable viruses, and ultrafiltration measurements which Tang and I [56] carried out in the laboratory last year placed the magnitudes of staphylococcus bacteriophage, herpes virus and the Rous sarcoma agent at about the same size—between 20 and 100 millimicrons. These measurements correspond with those of Olitsky and Boez [57] on the foot and mouth virus and suggest that they were dealing with a nonliving substance. This would stand somewhere in the scale of biologic complexity midway between the smallest known cells and the enzymes. Fascinating as this idea appears, and worthy of the most careful study, one must be on guard not to let its attractiveness carry one beyond present facts. There is no way at the present time by which it can be definitely shown either for the bacteriophage or for these viruses whether they are living or not, and no crucial experiment is possible. While the idea is consistent with almost all the clinical and experimental observations that have been made on herpes and on

78. Carrel, A.: Ann. Surg. **81**:1, 1925; Compt. rend. Soc. de biol. **92**:1493, 1925; **93**:1083, 1278, 1925; **94**:337, 1926; **96**:1121, 1927; Mechanism of Formation of Sarcoma, J. A. M. A. **84**:1795 (June 13) 1925.

encephalitis, it is impossible to harmonize it with observations on spontaneous transmission from patient to contacts or attendants. One of the most important points for investigation during the next outbreak, therefore, will be this question of spontaneous transmission, which is more complicated than is ordinarily supposed. Subjection to similar external conditions has often simulated direct transmission from person to person. This difficulty for a long time complicated the problem of pellagra, and I believe is confusing the problem concerning the common cold. Moreover, should herpes and encephalitis truly turn out to be secondary diseases, transmission of the encephalitic condition might well be simulated by the transmission of the primary condition which, in a small percentage of infected persons, would engender the secondary disease, as, for instance, in an epidemic of influenza in which the encephalitic complication occurs.

Notes and News

Meeting of American Association of Pathologists and Bacteriologists.—The next meeting of the American Association of Pathologists and Bacteriologists will be held in Chicago, March 28 and 29, 1929. The officers for the ensuing year are: president, E. B. Krumbhaar; vice president, George H. Whipple; secretary, Howard T. Karsner; treasurer, F. B. Mallory; member of council, Ward J. MacNeal.

University News, Promotions, Resignations and Appointments.—Grace Lubin succeeds R. L. Kahn in charge of the serologic work of the Michigan State Department of Health, Lansing.

It is reported that A. Schmincke has been appointed professor of pathology in the University of Heidelberg.

At the University of North Carolina, the department of pathology and bacteriology has been divided into two departments, pathology and bacteriology. J. B. Bullitt continues as professor of pathology and D. A. MacPherson assumes charge of the new department of bacteriology.

Martin H. Lovell has been appointed assistant in pathology at Meharry Medical College and resident pathologist to the Hubbard Hospital, Nashville.

Jacques J. Bronfenbrenner has been appointed professor and head of the department of bacteriology and immunology in Washington University Medical School, St. Louis.

At the University of Michigan, Malcolm H. Soule has been promoted to assistant professor of bacteriology.

Jeoffrey Hadfield has been appointed to the chair of pathology in the London School of Medicine for Women.

The University of Southern California is seeking endowment for its new medical school which will open soon. The appointments of professors in the preclinical departments are to be made within the next few months.

F. d'Herelle, director of the bacteriologic laboratory of the quarantine service at Alexandria, Egypt, has been appointed professor of bacteriology at Yale University.

William Late Coplin, emeritus professor of pathology and bacteriology in the Jefferson Medical College, died on May 29, at the age of 63. Dr. Coplin received his degree in medicine from Jefferson Medical College in 1886; he was professor of pathology and bacteriology in Vanderbilt University in 1895-1896 and in Jefferson Medical College from 1896-1922. He was acting assistant surgeon, U. S. Marine Hospital Service from 1890-1893, and he was director of the Department of Public Health and Charities in Philadelphia during 1905-1907.

Abstracts from Current Literature

Pathologic Physiology

A Familial Hemorrhagic Condition Associated with Prolongation of the Bleeding Time. George R. Minot, Am. J. M. Sc. **175**:301, 1928.

Five cases of a chronic familial hemorrhagic condition are described. The condition is characterized by a prolonged bleeding time in the absence of a decrease of blood platelets and without a delayed coagulation time, being thus distinguished from hemophilia and thrombopenic purpura. Ecchymoses, epistaxis and abnormal bleeding time are intermittent and not necessarily synchronous. The condition appears in infancy and tends to decrease in severity with time.

S. D. Simon.

Atypical Pathologic Hemorrhage in Early Life. Thomas E. Buckman, Am. J. M. Sc. **175**:307, 1928.

Five cases of purpura in infants, two of them in the same family, are described. Both bleeding time and coagulation time were prolonged, although the platelets were normal quantitatively and (in the one case so tested) qualitatively. The cause of the dyscrasia was not discovered.

S. D. Simon.

The Effect of Thyroidectomy on Spontaneous Activity in the Rat. M. O. Lee and E. F. Van Buskirk, Am. J. Physiol. **84**:321, 1928.

In a study of the effect of thyroidectomy on the spontaneous activity of adult white rats, continued over a period of three months, a change in the average daily activity was not observed. There was no evidence of lethargy, although the rats showed definite symptoms of hypothyroidism. The feeding of thyroid substance to a number of young adult female rats caused a slight decrease in spontaneous activity. During the estral cycle the changes in basal metabolic rate did not correspond with those of spontaneous activity. Castration or spraying greatly reduced the activity, but had only a slightly depressing effect on basal metabolism. A relation between the two phenomena was not evident.

H. E. Eggers.

Effects of Thymus, Muscle and Pituitary Extracts on Normal and Thyroparathyroidectomized Dogs. E. Larson and N. F. Fisher, Am. J. Physiol. **84**:330, 1928.

Extracts of calf thymus and muscle prepared in a manner similar to that used in preparing active parathyroid extracts showed a slightly ameliorative effect in acute parathyroid tetany. These, as well as pituitary extracts, did not affect blood calcium content sufficiently to explain this action. A parathyroid-like principle could not be demonstrated in the extracts used.

H. E. Eggers.

Some Effects of Early Castration on the Growth of the Male Rat. G. Van Wagenen, Am. J. Physiol. **84**:461, 1928.

Male albino rats castrated at the time of weaning fall behind their normal brothers in weight and body length. This lag in growth shows most strikingly and with increasing divergence after the first 100 or 150 days. Body length rather than total length afforded the significant measurement.

H. E. Eggers.

GROWTH RESPONSE TO ANTERIOR HYPOPHYSEAL EXTRACT BY THE CASTRATED MALE RAT. G. VAN WAGENEN, Am. J. Physiol. **84**:468, 1928.

The injection of the extract of the anterior portion of the hypophysis caused, in rats castrated in early life, an increase in body length equal to that of the controls, and an increase in weight in most cases considerably in excess of that of the controls. The total length of the injected castrated animals was greater than that of the controls, owing to excessive growth of the tails.

H. E. EGGERS.

THE SYNDROME OF MALIGNANT HYPERTENSION. NORMAN M. KEITH, HENRY P. WAGENER and JAMES W. KERNOHAN, Arch. Int. Med. **41**:141, 1928.

In cases of sustained high pressure and diffuse changes in the arterioles, the course is usually rapidly fatal owing to the simultaneous failure of brain, heart and kidneys. Diffuse changes in the arterioles are visible in the retina in life and are found in the tissues generally. The increased thickness of the intima and the hypertrophy of the elastic fibers and media make a characteristic picture.

SUSCEPTIBILITY TO HISTAMINE AS A TEST OF ADRENAL DEFICIENCY. W. J. M. SCOTT, J. Exper. Med. **47**:185, 1928.

The resistance of rats to histamine is greatly diminished after suprarenalectomy. This susceptibility to histamine is proposed as a functional test for deficient function of the suprarenal cortex.

AUTHOR'S SUMMARY.

A STUDY OF THE MECHANISM OF NUCLEINATE-INDUCED LEUCOPENIC AND LEUCOCYTIC STATES, WITH SPECIAL REFERENCE TO THE RELATIVE RÔLES OF LIVER, SPLEEN AND BONE MARROW. CHARLES A. DOAN, LEON G. ZERFAS, SYLVIA WARREN and OLIVIA AMES, J. Exper. Med. **47**:403, 1928.

The leukopenia induced by sodium nucleinate has been followed by repeated counts, made simultaneously from the blood of a peripheral vein and from the internal organs, combined with a study of histologic sections of the same organs taken with the counts. Measurements with the oncometer of changes in volume of the spleen have been correlated with the leukopenia and the leukocytosis following sodium nucleinate. It has thus been determined that the leukopenia is not the result of a vasomotor phenomenon, or of a change in blood volume, nor is it secondary to a retention of the white cells in the capillaries of the lung or the liver; it is due to the accumulation of neutrophilic leukocytes in the parenchyma of the spleen. That the spleen is solely responsible for the temporary depression of white cells in the general circulation under these conditions has been shown by splenectomy. In splenectomized rabbits leukopenia did not develop, but instead a leukocytosis due to a direct action on the bone marrow. A profound change occurs in the distribution of the cells in the circulation at the moment of death. The liver, within a minute of the cessation of the circulation, shows a three-fold to four-fold increase in the number of white cells per cubic millimeter, the differential count remaining unchanged. Thus, estimations of the physiologic distribution and redistribution of cells in the living state may be made only with the circulation unimpaired. The injection into normal rabbits of adenine and guanine nucleotides, split products of nucleic acid, caused immediate leukocytosis, originating in the bone marrow, similar to that observed with the more complex molecule when the spleen was eliminated. The response of the bone marrow to chemotactic stimuli, such as those here used, may be reflected in the general circulation, through an absolute increase of young neutrophilic leukocytes, within a period of less than one hour. Within this brief period maturation from myelocyte C and metamyelocyte into the early motile leukocyte occurs, and these just matured cells are delivered into the circulation. The response to one injection of nucleinate

or nucleotide may persist into the third and fourth days, with a gradual depletion of the normal reserve of myelocytes C in the bone marrow.

AUTHORS' SUMMARY.

EXPERIMENTS ON THE EFFECTS OF INSULIN. ARNDT, Verhandl. d. deutsch. path. Gesellsch. **22**:215, 1927.

The morphologic effect of insulin, the increase or decrease of glycogen, depends on the dose, the species of animal used and the condition of metabolism. Insulin injected into pregnant dogs and rabbits causes a decrease of the sugar in the maternal as well as the fetal blood. Of the organic glycogen of the fetus only that of the liver is affected. Small amounts of insulin injected into dogs daily for several months produced in the first months an increase in weight; a period in which the weight remained stationary followed, and finally a marked decrease in weight resulted, with hypersensitiveness of the organism to small doses of insulin, demonstrated by a considerable drop of the blood sugar. The animals died during an attack of hypoglycemia. Rabbits similarly treated did not show these effects. Insulin given in one large dose or in repeated small doses to dogs poisoned with phosphorus did not have any effect on the fatty degeneration of the liver. If sugar was added to the diet, the formation of glycogen in the liver and an absence of the fatty degeneration was observed. Experiments with synthalin given in small doses showed an increase of glycogen in the liver during starvation. The administration of large doses, however, resulted in a marked decrease or complete disappearance of the glycogen.

WILLIAM C. HUEPER.

Pathologic Anatomy

THE PHARYNGEAL TONSIL. W. E. COOKE, Am. J. Dis. Child. **35**:229, 1928.

The pharyngeal tonsil begins to develop about the fourth month of fetal life and is a fully functioning organ by the time the child is viable. Histologically, it is a lymphoid organ that secretes mucus and is arranged in crypts with a surface covering of ciliated columnar epithelium. It probably functions as a filter for inspired air. Bacteriologic studies reveal the same flora as ordinarily inhibit the nasopharynx.

The most important pathologic condition is hypertrophy, the cause of which is not definitely understood, but which consists of hyperplasia of the lymphatic elements with a change in the normal epithelium to the stratified type. Sometimes cysts are formed by the occlusion of crypts, and the epithelial lining undergoes mucoid degeneration. Certain pressure effects of the lymphatic mass are described, among which are (1) the formation of concentric cell masses called "cell nests"; (2) fibrosis of the basement membrane and (3) hyperplasia of the reticular tissue of the germinal centers. Formation of abscesses is described. As adolescence approaches, the organ diminishes in size by decrease in the number of germinal centers.

PEARL M. ZEEK.

COEXISTING EXTRAUTERINE AND INTRAUTERINE PREGNANCY. A. STEIN, Am. J. Obst. & Gynec. **15**:159, 1928.

In a nullipara, aged 29 years, pregnant for the third time, there was found at operation, which was done in December, a left-sided tubal mass the size of a grape fruit, which on histologic examination was found to be composed of blood clot and chorionic villi. Incision of the moderately enlarged uterus was followed by the escape of the placenta and a two and one-half months' fetus. The extra-uterine pregnancy was believed to have taken place in May and the intra-uterine pregnancy in October preceding operation. From the literature, Stein adds reports of 36 cases of such combined pregnancies to the 243 gathered by Neugebauer in 1913.

A. J. KOBAK.

CHANGES IN THE SEDIMENTATION RATE OF THE ERYTHROCYTES AND IN THE PLASMA PROTEINS FOLLOWING PROLONGED CHLOROFORM ADMINISTRATION TO THE DOG. M. D. ROURKE and E. D. PLASS, Am. J. Physiol. **84**:42, 1928.

In four dogs which had been poisoned by long administration of chloroform, the resulting necrosis of the liver was found associated with marked reduction of plasma fibrinogen and of rate of red cell sedimentation. The two apparently were associated. Bile pigments of plasma and urine and plasma lipoids showed relative increases which corresponded to the changes in the liver as indicated by the reduction of fibrinogen and of sedimentation rate. There was little variation in the albumin and globulin fractions of the plasma.

H. E. EGGERS.

AN EXPERIMENTAL INVESTIGATION INTO THE EFFECTS OF ASPHYXIA ON THE BRAIN, WITH ESPECIAL REFERENCE TO ASPHYXIA NEONATORUM. FRANK R. FORD, Bull. Johns Hopkins Hosp. **42**:70, 1928.

Lesions of the brain could not be produced in kittens by experimental asphyxia. These results support those of Hannes and others indicating that asphyxia of the new-born is not, as widely believed, a cause of cerebral injuries at birth. The results of other experiments indicate that the cerebral lesions in poisoning with illuminating gas are not due solely to carbon monoxide.

TUBERCULOUS ANEURYSM OF THE HEPATIC ARTERY. W. P. THOMPSON, Bull. Johns Hopkins Hosp. **42**:113, 1928.

A case is presented in which an aneurysm of the hepatic artery was apparently produced by an adjacent tuberculous process.

AUTHOR'S SUMMARY.

THE ETIOLOGY OF PYELITIS IN PREGNANCY. J. HOFBAUER, Bull. Johns Hopkins Hosp. **42**:118, 1928.

Urinary obstruction in pregnant women is caused by certain anatomic conditions in the juxtavesical portion of the ureter and in the trigonum vesicae. Hypertrophic changes in the musculature associated with hyperplastic changes in the connective tissue are essential factors in the narrowing of the lumen of the lower part of the pelvic portion of the ureter. The constriction is still further accentuated by an encircling ring resulting from hypertrophy of the ureteral sheath, while engorgement of the vessels in the mucosa and dextrorotation of the uterus may act as contributory factors. There was no demonstrable indication of an active inflammatory process or of remnants of a preceding inflammation in the ureteral wall in the specimens examined. A moderate degree of hydro-ureter is a common occurrence in pregnant women. The distal end of the ureteral dilatation usually lies at the level of the parametrium, the visible dilatation being associated with a demonstrable delay in ureteric action. The hyperplastic and hypertrophic changes in the upper pelvic portion and in the abdominal portion of the ureter are decidedly less marked, and the dilatation above the narrowed area of the juxtavesical portion occurs as a consequence of the structural peculiarities described in this paper. The hypertrophy of the trigonum accounts for the clinical phenomenon of residual urine in pregnant women. In seven out of fifty-five cases a definite lowering of the opsonic index of the serum toward *Bacillus coli* during pregnancy could be demonstrated. The bearing of these observations on the development of actual pyelitis is discussed. Histologic evidence tends to substantiate the occurrence of involution processes within the ureteral wall, in analogy with the phenomena occurring in the uterus during the puerperium. While a gradual return of the renal pelvis and of the ureter toward normal occurs after labor in uncomplicated cases, persistence of both bacteria and of marked dilatation of the ureter was demonstrable on reexamination in a considerable percentage of women who had been treated for pyelitis during a preceding pregnancy. In the vast majority of

these cases, the level above which the ureter has remained dilated corresponds to the parametrium. Stricture of the ureter may occasionally result from long-standing infection in the ureteral wall during pregnancy.

REGENERATION OF THE BLADDER FOLLOWING RESECTION. HERMAN L. KRETSCHMER and K. E. BARBER, J. A. M. A. **90**:355, 1928.

Extensive resection of the bladder is followed by the formation of a new bladder. The newly formed bladder completely fulfils the function of the old bladder in that it is capable of retaining the urine for many hours and of discharging urine in the normal manner. Incontinence as a permanent complication does not follow even the widest type of resection. From the histologic picture and its close resemblance to the normal bladder, it would appear that the newly formed bladder is the result of regeneration.

AUTHORS' SUMMARY.

PURPURIC SMALLPOX. KANO IKEDA, J. Lab. & Clin. Med. **13**:440, 1928.

The purpuric type of smallpox does not present an anatomic resemblance to the pustular type of this disease. Microscopically, identification may be difficult in the absence of early variolar lesions in the skin. Diffuse subcutaneous, submucous and subserous hemorrhages are the most striking feature of this type of smallpox. Intense streptococcic bacteremia is demonstrated in practically all of the cases studied. The blood picture, while probably not specific, is sufficiently characteristic in every case of purpuric smallpox to be considered of prime importance in its diagnosis and its differentiation from other purpuras.

AUTHOR'S SUMMARY.

LIVER INJURY IN ACUTE ALCOHOLIC POISONING. R. P. WALLACE, Proc. Soc. Exper. Biol. & Med. **24**:598, 1927.

Quantitative determinations of the bilirubin in the blood serum and of the urobilinogen in the urine were made, the first in each of seventeen patients with acute alcohol poisoning in Bellevue Hospital, New York, the second in only five of the seventeen. The bilirubin was found increased from one-fourth to three times over normal. The increase in urobilinogen ran parallel with that of the bilirubin, and their increase corresponded well with the severity of the poisoning. From these results it is concluded that alcohol damages the liver.

E. R. LE COUNT.

HISTAMINE AND LEUCOCYTE EMIGRATION. R. T. GRANT and J. EDWIN WOOD, JR., J. Path. & Bact. **31**:1, 1928.

Observations are described to show that histamine does not have an appreciable power to cause emigration of leukocytes from the blood vessels when injected intraperitoneally or applied to the exposed mesentery or the conjunctiva in the frog, when instilled into the conjunctival sac of the rabbit or when pricked into the human skin. It is concluded that release of histamine from injured tissue does not provide a full explanation of the process of inflammation.

AUTHORS' SUMMARY.

THE HISTOLOGY OF POSTVACCINAL ENCEPHALITIS. J. R. PERDRAU, J. Path. & Bact. **31**:17, 1928.

The claim of Turnbull and McIntosh that postvaccinal encephalitis can be distinguished histologically from epidemic encephalitis is confirmed. Although comparison with poliomyelitis could not be carried out satisfactorily, owing to lack of material, postvaccinal encephalitis and poliomyelitis are probably different

as regards the type of lesion. Postvaccinal encephalitis is characterized by the presence around certain vessels of the central nervous system of an area of demyelinization (the extra-advential infiltration or softening of Turnbull and McIntosh). From a study of the literature it appears that the nervous disorders complicating smallpox, measles and other fevers as well as those occurring in the course of antirabic inoculations (Pasteur's treatment) are histologically identical with postvaccinal encephalitis and myelitis. The perivascular demyelinization of postvaccinal encephalitis is essentially similar to that found in comparatively acute cases of disseminated sclerosis. The possibility is discussed that the agent (living or not) responsible for the demyelinization in these various forms of nervous disorder may be the same for all of them.

AUTHOR'S SUMMARY.

VARIATIONS IN THE LEUCOCYTE COUNT UPON SINGLE AND REPEATED INJECTION OF QUARTZ AND OF INDIAN INK PARTICLES. A. R. ELVIDGE, J. Path. & Bact. **31**:33, 1928.

The intravenous injection of quartz and of India ink causes an immediate and marked fall in the white cell count of the rabbit, which is usually followed by a period of leukocytosis. Both polymorphonuclear leukocytes and lymphocytes take part in the leukopenia and the succeeding phase of leukocytosis. The cells which remain in the circulation during the period of leukopenia are mostly lymphocytes; in some cases polymorphonuclear elements are entirely absent from the blood smear. Leukopenia does not necessarily cause a subsequent leukocytosis. Leukocytosis produced by the intravascular injection of particles is usually accompanied by a "blood crisis," but with suitable dosage the white cell reaction can be separated from the red cell reaction. Exceptional reactions toward single injections are occasionally observed in rabbits which show an abnormally high leukocyte count previous to experimentation. At least three types of reaction may be observed. Two of these can be produced in rabbits by repeated injections of particles. If a second dose is given during the leukocytosis, owing to the first dose, leukopenia does not follow. These reactions are nonspecific, being common at least to quartz and to India ink particles. The age of the white cell is apparently not the factor responsible for the difference in reaction toward repeated injections. Blood platelets capture ink particles more rapidly than do white cells. Only when the blood platelets have become loaded to capacity are the white cells affected.

AUTHOR'S SUMMARY.

PROLIFERATION OF ENDOTHELIUM ON THE LIVER IN EXPERIMENTAL VENOUS STAGNATION. CHARLES BOLTON and W. G. BARNARD, J. Path. & Bact. **31**:45, 1928.

Proliferation of the endothelium over the surface of the liver, occurring independently of inflammation and leading to the production of polyps, has been found in experimental venous obstruction.

AUTHORS' SUMMARY.

THE DISTRIBUTION OF TUBERCULOUS LESIONS IN MAN AND OTHER PRIMATES WITH AN ACCOUNT OF THE LYMPHATIC GLANDS AND VESSELS OF THE THORAX AND UPPER ABDOMEN. HENRY HAROLD SCOTT and JOHN BEATTIE, J. Path. & Bact. **31**:49, 1928.

Two distinct types of tuberculosis are to be found both in man and in other primates. The respiratory type is a rapidly fatal infection causing death by generalized dissemination of bacilli by the lymphatics and blood vessels. The alimentary (primary) form of the disease is less rapidly fatal. Alimentary tuberculosis may be secondary to a primary respiratory infection. The disease is transmitted to the intestine by the swallowing of large numbers of tubercle bacilli in sputum. Monkeys and apes never expectorate. In each type of the disease the dissemination of infection by the lymphatic system takes place in the same

order in monkeys as in man. The proportion of alimentary tuberculosis to respiratory tuberculosis is probably much the same in all primates. Respiratory tuberculosis is identical in young human subjects and in other primates, but in later life in the former a modifying factor comes into play. Tuberculosis in early human life is the "natural" form of the disease. In other primates the modifying factor is absent and the adults are affected in the same way as the young animals.

THE CELLS OF PERITONEAL EXUDATES IN GUINEA-PIGS. L. J. WITTS, J. Path. & Bact. **31**:101, 1928.

The supravital technic has been applied to peritoneal exudates. In peritonitis in the guinea-pig the cells of the exudate appear in the order serosal cells, neutrophils, lymphocytes, eosinophils and macrophages. Neither serosal cells nor lymphocytes are converted into macrophages. The macrophages are of the type of tissue phagocytes or clasmatocytes, and few typical monocytes can be recognized. It is probable that monocytes take on the characteristics of clasmatocytes on leaving the blood stream.

AUTHOR'S SUMMARY.

POSTARSPHENAMINE PURPURA. V. P. LESPINNE and FÉROND, Sang. **1**:193, 1927.

The injection of arsphenamine into the vein affects the blood in normal as well as in pathologic conditions. In normal blood the drug interferes with its coagulability and with the retraction of the clot. In purpuric patients, the introduction of derivatives of arsphenamine leads to a hemoclasic shock evidenced by hypoleukocytosis and inversion of the leukocytic formula. At times a persistent leukopenia occurs. A disappearance of hematoblasts, a destruction of red cells and a hypocoagulation or hypercoagulation also occur. Arsphenamine introduced into the blood produces a colloidal flocculation owing to the formation of a precipitate by the acids of the blood. The postmortem examination of the viscera does not show any lesions in the liver, kidneys or suprarenals. Marked changes are found in the spleen and in the bone marrow. The toxin or toxins leading to purpura affect the vascular endothelium which is focal in nature. The fact that in purpura the cutaneous vessels are affected is possibly connected with the fragility of these capillaries and also with some nervous disturbances.

B. M. FRIED.

PULMONARY EDEMA AND CRANIAL INJURIES. A. ANTONINI and A. BIANCALANI, Arch. di anthrop. crim. **47**:747, 1927.

The pathogenesis of pulmonary edema is essentially that of pulmonary hypertension, vasodilatation and transudation of serum. This is brought about by the spasm of the left ventricle resulting in a relative hypotension of the general circulation and the corresponding hypertension of the lesser circulation. This condition brings about a lack of balance in the function of the two ventricles, leading to distention of pulmonary capillaries. In addition to this, there is a nervous theory, a toxic theory and a mixed theory to explain this phenomenon. The authors have made postmortem examinations in 200 cases of craniocerebral lesions. The intermediary period between the injury and death varied from a fraction of one hour to three days. Pulmonary edema was present in 82, or 41 per cent of the patients. Without going into a discussion of all the facts, it is possible to state that pulmonary edema takes place in a varying number of cases of craniocerebral injuries in which a certain period between the injury and death exists. Histologic examination of various organs, such as the myocardium, kidneys and aorta failed to shed any light on their participation in this process. The preexistence of organic lesions favoring the phenomenon could not be suspected in young persons and in infants. In all his cases Moutier has found an increase of the systolic pressure with a full and strong pulse which is in great contrast with the grave condition of the patient. This is followed by an increase

in diastolic pressure and diminution in the differential pressure. The authors regret not to have had the opportunity to make clinical observations in their cases. Modern idea of shock and of states related to shock is summed up in the rôle played by histamine. Histamine is capable of increasing arterial pressure. It has a vasoconstricting effect on the arteries and a vasodilating effect on the arteries. Thus in the absence of organic cardiac and renal changes the hypothesis of a toxic factor finds its application.

THE REACTIONS OF LYMPHATIC NODES TO VARIOUS DYES INJECTED INTRAVITALLY. J. S. LATTA and R. Z. SCHULZ, Folia. Haemat. **35**:119, 1927.

The authors stained white rats with vital dyes "to bring about such reactions in the lymphatic nodes as would be in some degree, similar to those resulting from bacterial invasion." This, then, would help them to interpret the rôle of these organs in the defense of the body against invasion. In addition to sinus macrophages, the dye was found abundantly in the cytoplasm of the fixed cells which lined the sinuses. Interestingly enough, the number of macrophages which was found within the lymph sinuses varied according to the response of the cells which lined the sinuses to the toxicity of the dyes used. The increase in free wandering macrophages was due chiefly to the transformation of the lining cells rather than to any proliferative activity of these cells. The small lymphocytes, too, which under normal conditions never store dye, "do when sufficiently exposed to the toxic influence of a dye or to other catabolic agents, differentiate into cells which are classed as macrophages, capable of storing dye or granules." In general, the accumulation of the dye in macrophages depended on their degree of degeneration, the cell becoming more permeable to the dye as its degeneration continued. "Phagocytosis," according to the authors, "would be considered as a physical sign of a degenerative condition of the cells, and a process in which they were entirely a passive agent."

B. M. FRIED.

ZYTOLOGISCHE UNTERSUCHUNGEN VON ERGÜSSEN DER BRUST- UND BAUCHHÖHLEN MIT BESONDERER BERÜCKSICHTIGUNG DER KARZINOMATÖSEN EXSUDATE. ULRIK QUENSEL. Pp. 183 and 49 plates with 99 figures. Uppsala och Stockholm: Almquist & Wiksell, 1928.

Examination by the usual cytologic methods did not differentiate sufficiently between the different cells that may occur in pleural and peritoneal exudates. A more satisfactory differentiation was obtained by staining the moist sediment with methylene blue-cadmium and sudan-cadmium. In the exudates that form in malignant processes, red corpuscles, lymphocytes, leukocytes, endothelial cells and, in most cases, specific tumor cells may occur, but in varying numbers in different cases. While the tumor cells are not always distinguished easily from other cells, especially endothelial cells, they often present differentiating characteristics. In most of the cases of carcinomatous exudates examined by Quensel, he was able to recognize carcinoma cells, but there is no general cytologic formula that can be applied to all cases because the number and form of the tumor cells vary greatly. The cytologic picture depends on the nature of the primary tumor and of the carcinomatous process in the serous cavity. In scirrhous carcinoma, the number of tumor cells in the exudate is small or the cells may be absent altogether. In the medullary forms, however, there is a larger number of cells in the exudate, especially when the growth extends over the serous surface. The tumor cells may occur singly or in smaller and larger groups. In the latter case, they occur mostly in the form of round masses with sharp borders in which the cells are distributed in different levels. On the other hand, endothelial cells occur more commonly in thin sheets in which the cells lie in the same level. The tumor cells are frequently larger and often stain more deeply than the endothelial cells. The nucleus of the tumor cell is often unusually large and the nucleolus may be large, irregular in form and sometimes increased in number. The presence

of fat and vacuoles in the cells of exudates is not characteristic of tumor cells by itself, but very large, giant vacuoles speak in favor of the carcinomatous nature of the cells.

Pathologic Chemistry

THE EFFECT OF INSULIN ON THE LIPIDS OF RABBIT LIVER. E. R. THEIS, J. Biol. Chem. **77**:75, 1928.

The administration of insulin causes a rapid and decided decrease in the proportion of phospholipid to neutral fat in rabbit liver tissue. Continued administration causes a decrease in the total lipid content. The insulin effect on the phospholipin-neutral fat ratio reaches its maximum coincidentally with the occurrence of convulsions.

ARTHUR LOCKE.

THE EFFECT OF INSULIN ON THE AMINO-ACID CONTENT OF BLOOD. J. M. LUCK, G. MORRISON, and L. F. WILBUR, J. Biol. Chem. **77**:151, 1928.

The administration of subconvulsive doses of insulin to rabbits, rats and men produces a decrease in the amino-acid content of the blood which is, roughly, 80 per cent as great as the accompanying effect on the sugar concentration. The decrease is an effect of insulin and not a consequence of the induced hypoglycemia.

ARTHUR LOCKE.

INORGANIC FACTORS DETERMINING CALCIFICATION. D. H. SHELLING, B. KRAMER, and E. R. ORENT, J. Biol. Chem. **77**:157, 1928.

Shipley's observation on the calcification of rachitic cartilage when incubated in normal rat serum supplied a new method for the study of calcification. The remarkable localization of the calcification in vitro and its failure to occur when the cells have been injured by protoplasmic poisons, at once differentiate this process from that which occurs in pieces of dead cartilage, whether in vivo or in vitro, and prove that the process is similar, in some respects, at least, to that occurring in vivo. Two sets of conditions may effect calcification: (1) conditions affecting the cartilage cells themselves, and (2) changes in the physio-chemical composition of the solution bathing the cells. Hitherto, the latter has been the point of attack. The composition of the solution has been modified in various ways, and the effect on calcification in vitro has been studied. We have shown that the reaction of the solution is one factor that determines calcification. There is an optimal reaction, that of normal blood serum. An increase of the total ionic strength of the solution, whether produced by increased amounts of sodium chloride or potassium chloride, inhibits calcification. This inhibition occurs at lower concentrations of sodium chloride or potassium chloride if the concentrations of calcium and phosphate are also lower. Calcification occurs more readily in the absence of magnesium. The inhibitory effect of magnesium can be overcome by the addition of suitable amounts of phosphate, the mechanism of which cannot be explained at present. This inhibitory effect may be due either to the toxic effect of magnesium ion on cartilage cells, or to the formation of a unionized magnesium compound.

AUTHORS' SUMMARY.

THE NATURE OF THE MATERIAL IN LIVER EFFECTIVE IN PERNICIOUS ANEMIA. E. J. COHN, G. R. MINOT, G. A. ALLES and W. T. SALTER, J. Biol. Chem. **77**:325, 1928.

Liver contains a substance which effectively increases the concentration of reticulocytes and erythrocytes in persons having pernicious anemia. The substance appears to be soluble in water, insoluble in ether and precipitable by alcohol. It is not precipitable by basic lead acetate or phosphotungstic acid. It contains no iron, and is not protein, carbohydrate or lipoid.

ARTHUR LOCKE.

DETERMINATION OF BILE ACIDS IN THE BLOOD. M. ALDRICH and M. S. BLEDSOE, J. Biol. Chem. **77**:519, 1928.

A method is described for the colorimetric approximation of the bile acid content of whole blood. The technic is simple, only 5 cc. of blood is required, and the results appear to indicate the presence of bile acids (in amounts of from 0.1 to 0.5 mg.) with an accurracy of ±5 per cent. Normal blood has a bile acid concentration corresponding with from 3 to 6 mg. of glycocholic acid per hundred cubic centimeters.

ARTHUR LOCKE.

QUANTITATIVE ESTIMATION OF CAROTIN IN BLOOD AND TISSUES. C. L. CONNOR, J. Biol. Chem. **77**:619, 1928.

The method suggested is a modification of that proposed by van den Bergh, Muller and Broeckmeyer. Carotin is a normal constituent of the blood and of blood-containing organs. It occurs in greatest concentration in the suprarenal glands (in adult rabbits and guinea-pigs), is present in the liver and corpus luteum, and in the fat. It is not present, in demonstrable amounts, in the heart or seminal vesicles. The concentration of carotin in the blood of persons with diabetes is slightly higher than that found in the blood of normal persons.

ARTHUR LOCKE.

THE ESTIMATION OF UREA AND AMINO-ACID NITROGEN IN ANIMAL TISSUES. C. KIECH and J. M. LUCK, J. Biol. Chem. **77**:723, 1928.

A method is described for the estimation of amino-nitrogen and urea in animal tissues. The latter is determined gravimetrically as dixanthydryl urea by direct weighing. Tungstic acid is employed as a protein precipitant.

AUTHORS' SUMMARY.

STUDIES IN THE PHYSICAL PROPERTIES OF DIFFERENT BLOOD SERA. 1. ISO-ELECTRIC POINTS OF BLOOD SERA AND THEIR SIGNIFICANCE IN THE ANTIMONY TEST FOR KALA-AZAR. R. N. CHOPRA and S. G. CHAUDHURI, Indian J. M. Research **15**:895, 1928.

The iso-electric points of globulin and albumin in normal and pathologic human serums are 5.5 and 3.8, respectively. In some lepers and persons with kala-azar, the iso-electric point of albumin is 4. The precipitate formed by the addition of a solution of urea-stibamine is mainly euglobulin. The antimony test for kala-azar can be explained on the basis of: (1) absence of change in the iso-electric points, (2) increase in the globulin-albumin ratio, and (3) diminution of buffer action.

AUTHORS' SUMMARY.

INTRACELLULAR HYDROGEN ION CONCENTRATION. M. SCHMIDTMANN and A. NUREDDIN-SEKI, Ztschr. f. d. ges. exper. Med. **58**:340, 1927.

(Abstracts of previous papers on this subject may be found in *Arch. Path.* **5**: 713 and 714 [April] 1928). The experiments were designed to decide the question whether cells contained as effective a buffer system for maintaining constant hydrogen ion concentration as that which exists in the blood. Kidneys or livers of rabbits were surgically removed and perfused with oxygenated Ringer and Locke's solution; a p_H range of from 3.2 to 8.6 was obtained by the addition of lactic acid or acetic acid, and of sodium bicarbonate or sodium carbonate. The intracellular p_H was determined from excised pieces of tissue by the methods previously described (reference should be made to the first abstract of the series). Criteria for death of cells were: (1) permeability of the nuclear membrane to vital dyes, and (2) shifting of intracellular reaction to that of the medium. According to these criteria, perfusion with Ringer's solution did not lead to death of cells during the time of the experiments. Perfusing fluids of p_H below 5.0 rapidly led to death

of cells. Solutions of less acidity did not kill cells; a slight, but definite, change of their reaction was obtained. The cells became swollen, granular or vacuolated, and lipoid substances became visible. These morphologic changes appeared before demonstrable changes occurred in intracellular reaction. Perfusion with alkaline solutions led to a certain degree of shifting of the cellular p_H. Prolonged perfusions had an injurious effect; the cell borders and the nuclear membranes became more distinct, and the cytoplasm more transparent. These experiments suggest that the morphologic appearance of the cell is largely determined by the reaction of the medium, and that the intracellular hydrogen ion concentration can be altered, within narrow limits.

B. LUCKÉ.

ON THE POTASSIUM AND CALCIUM CONTENT OF THE BLOOD AND ORGANS OF RABBITS AND DOGS, AND ITS VARIATION IN SENSITIZED AND ANAPHYLACTIC ANIMALS. A. SCHITTENHELM, W. ERHARDT and K. WARNAT, Ztschr. f. d. ges. exper. Med. **58**:662, 1928.

The level of potassium and calcium varies somewhat in different organs and in different parts of the vascular system (carotid artery and portal, hepatic and jugular veins). During the period of sensitization there occurs a slight increase of potassium in the blood serum. In acute anaphylactic shock there is a sudden marked rise of potassium in the serum; this is most pronounced in the portal vein and least prominent in the carotid artery. In prolonged anaphylactic shock the serum potassium of the portal vein is even higher than in acute shock; the values of potassium in the carotid artery are lower than in acute shock; in the hepatic vein the increase is less marked than in the portal. It appears that potassium is retained, to a certain extent, by the liver. There is a certain rise in the level of serum calcium, which is interpreted as an attempt to equalize the calcium potassium ratio. It is possible that the sudden liberation of potassium (from the erythrocytes [?] and tissue cells) is in part responsible for the circulatory phenomena of anaphylactic shock. The potassium content of whole blood is normally seven times greater than that of serum; during anaphylactic shock, potassium increased in the serum, but not in whole blood. In the organs (liver, lung, spleen, intestines) of exsanguinated anaphylactic animals the potassium content is reduced to about one-half its normal value.

B. LUCKÉ.

PHYSIOLOGIC CHEMISTRY OF TISSUES WITH RELATION TO AGE. M. BÜRGER and G. SCHLOMKA, Ztschr. f. d. ges. exper. Med. **58**:710, 1928.

This is the second paper of this series; the first study (*Ztschr. f. d. ges. exper. Med.* **55**:287, 1927), dealt with human cartilage. Eyes were obtained from a municipal slaughter house; the ages of the animals varied from less than 1 year to 17 years. The material (lens) was examined for content of water, nitrogen, cholesterol and calcium. It was found that the water content steadily declined (from 68.5 per cent during the first year to 63.4 per cent during the fifteenth to seventeenth year); while the nitrogen content steadily rose (from 5.04 per cent to 5.89 per cent). Cholesterol contents increased from 40 mg. per hundred cubic centimeters in new-born calves to 222 mg. per hundred cubic centimeters in 15-year-old cattle (or, expressed as a percentage of the dried lens, from 134 mg. per hundred cubic centimeters to 610 mg. per hundred cubic centimeters). Only traces of calcium were found in the tissues of normal lenses.

B. LUCKÉ.

Microbiology and Parasitology

SURVIVAL OF LEPTOSPIRA ICTEROIDES IN VARIOUS ENVIRONMENTS. W. A. SAWYER and J. H. BAUER, Am. J. Trop. Med. **8**:17, 1928.

By inoculating various specimens of sterilized water from stagnant pools with *Leptospira icteroides* and testing them at varying times for the presence of the organism, it was found that *Leptospira icteroides* may survive in the water for

as long as fifty-five days. By testing with immune serum the surviving organism was proved to be the same as the one inoculated.

Leptospira icteroides was also isolated from freshly inoculated feces, but it died out rapidly in fecal material.

The organism was not recovered from inoculated urine, although large numbers were found in the urine of two of twelve guinea-pigs which died from infection with *Leptospira icteroides.* Cultures were made from mosquitoes at varying times after they had fed on infected guinea-pigs. Nine hours was the longest time *Leptospira icteroides* survived in *Aedes aegypti.*

PEARL M. ZEEK.

EXPERIMENTAL TRANSMISSION OF YELLOW FEVER TO LABORATORY ANIMALS. ADRIAN STOKES, JOHANNES H. BAUER and N. PAUL HUDSON, Am. J. Trop. Med. **8**:103, 1928.

The virus of yellow fever is found to produce in monkeys of the species *Macacus rhesus* a clinical and pathologic picture similar to that produced by the virus in human beings. The disease is transmissible from man to monkey as well as from monkey to monkey by the injection of citrated blood or serum taken early in the disease. It is also transmissible by mosquitoes of the species *Aedes aegypti.* The virus is filtrable when in the circulating blood but when in the mosquito it fails to pass under high pressure through the coarsest grade of Berkefeld filters, thus indicating a probable difference in size and morphology when in this host.

Convalescent serum from a severe case of yellow fever, in doses of 0.1 cc., protects monkeys against infection with this virus, while 2 cc. of normal human serum fails to give any protection. Mosquitoes, when once infected, are found to remain infective for the entire period of their lives, which may exceed three months. The bite of a single infected mosquito is sufficient to produce a fatal infection in a monkey.

PEARL M. ZEEK.

SURFACE COLONY TYPES OF CL. BOTULINUM ON BLOOD AGAR. P. SCHOENHOLZ, J. Infect. Dis. **42**:40, 1928.

Five different surface colony types of *Clostridium botulinum* on veal infusion sheep blood agar plates have been distinguished. *Cl. botulinum* varies in its hemolytic properties. Purified cultures of *Cl. botulinum* may consist of more than one colony type. The colony types can be isolated in pure culture. One type, the variant, breeds true over long periods; the other types isolated from the stock cultures, considered parent strains, may be obtained in pure form, but when allowed to stand at room temperature, the variant may be isolated again. Nontoxic variants have not been encountered.

AUTHOR'S SUMMARY.

EFFECT OF CONCURRENCE ON TYPHUS AND ROCKY MOUNTAIN SPOTTED FEVER INFECTIONS IN GUINEA-PIGS. F. BREINL, J. Infect. Dis. **42**:48, 1928.

The viruses of typhus and Rocky Mountain spotted fever injected in equal parts and simultaneously into the guinea-pig do not exert any apparent influence on each other, and each produces active immunity.

Preceding infection with typhus virus has a mitigating influence on an infection with spotted fever when the superinfection takes place during or immediately after the fever from the typhus.

Preceding infection with spotted fever renders the guinea-pig insusceptible for small doses of typhus virus injected on the first day of fever. Larger doses of typhus virus inoculated on the same date take, but the following disease runs a mild course. The reduction of the susceptibility is transitory and nonspecific.

AUTHOR'S SUMMARY.

ON THE SPECIFICITY OF SCARLET FEVER STREPTOCOCCI. S. I. ZLATOGOROFF and W. S. DERKATCH, J. Infect. Dis. **42**:56, 1928.

Streptococci derived from sources other than scarlet fever produce toxic substances toxins. These substances cause a skin reaction of the Dick type under the same conditions as the toxins of scarlatinal streptococci.

Substances producing the phenomenon of extinction (Schultz-Charlton) may be obtained by immunization with scarlatinal as well as nonscarlatinal streptococci.

A positive Dick reaction may be obtained during the period of scarlet fever complications with scarlatinal as well as nonscarlatinal streptococci. This fact brings one to suppose in this case a reaction to the unspecific bacterial proteins.

Scarlatinal serums give the reaction of flocculation (precipitation) with toxins of streptococci derived from various pathogenic sources.

Neither the toxin production nor the Schultz-Charlton test enables one to confirm the conception of the specificity of scarlet fever streptococci.

AUTHORS' SUMMARY.

STUDIES ON THE METABOLISM OF THE ABORTUS-MELITENSIS GROUP. JAMES G. MCALPINE and CHARLES A. SLANETZ, J. Infect. Dis. **42**:66 and 73, 1928.

By the methods employed in this study, it has been shown that *Bacterium abortus* of bovine origin utilizes little or no dextrose in its metabolic activity. On the other hand, *Bact. abortus* of porcine and human origin and *Bact. melitensis* consumed from 4 to 18 per cent of this carbohydrate for growth energy.

Because of this difference in sugar metabolism, *Bact. abortus* bovine can be differentiated from *Bact. abortus* porcine and human, and from *Bact. melitensis* by the different amounts of the various nitrogen fractions present in the culture medium over an incubation period of fourteen days. This difference is apparent only in dextrose-containing mediums.

Carbon dioxide, 10 per cent, stimulates the growth of *Bact. abortus* bovine, even though the strains may have become accustomed to aerophilic conditions, but it partially inhibits the multiplication of *Bact. abortus* porcine and human, and *Bact. melitensis*. This inhibition may be due to a slight change in the hydrogen ion concentration caused by the carbon dioxide and perhaps partly to a decrease in oxygen supply in the closed, as compared with the open, culture system.

Quantitative sugar determinations made by the Somogyi and Benedict methods, and p_H determinations according to the colorimetric method of Clark, when Fairchild's peptone is employed in the medium show that the *Bact. abortus melitensis* group may be split into two main parts. The first of these includes all strains which are unable to utilize more than 2 per cent of dextrose. The second group includes those which use from 5 to 20 per cent of the carbohydrate and is made up of *Bact. abortus* of human and porcine origin, and *Bact. melitensis*. These results were consistent with a large number of strains, barring one exception. This was a bovine strain which showed from 8 to 10 per cent utilization. It is not unlikely that in some instances cows become infected with the porcine strain of the organism.

All the strains from human beings were apparently more closely related to the porcine strains than they were to those of bovine origin.

AUTHORS' SUMMARY.

THE SUCCESSFUL CULTIVATION OF THE GONOCOCCUS ON BLOOD AGAR PLATES. RUSSELL D. HERROLD, J. Infect. Dis. **42**:79, 1928.

Blood is more valuable as an enrichment for nutrient solid mediums for isolating gonococci if from 0.75 to 1 per cent agar is used. The value of this medium is further enhanced by the use of phosphate instead of sodium chloride and the addition of blood (from 10 to 15 per cent) to the agar at 65 C. followed by gradual cooling to 45 C. before it is poured into plates or slants. Nutrient solid mediums thus prepared have proved valuable in the isolation of gonococci from both acute and chronic infections.

AUTHOR'S SUMMARY.

A Comparison of B. Chauvoei Strains from Cattle and Sheep. Erwin Jungherr, J. Infect. Dis. **42**:84, 1928.

In foreign countries, the majority of blackleg-like diseases in sheep have been found to be due to *Bacillus chaeuvoei*. Bacteriologic investigations showed that consistent differences cannot be made out between bovine and ovine strains of *B. chauvoei*. On the other hand, certain biologic differences seemed to deny any interrelationship of bovine and ovine blackleg. In this country, blackleg in sheep as a field condition has been definitely reported from Montana. The strains isolated from these field outbreaks have been diagnosed as *B. chauvoei*. Unpublished results of sheep-inoculation experiments conducted by the Montana Experiment Station indicate a wide variation of virulence among *B. chauvoei* strains of either bovine or ovine origin.

In the comparative laboratory studies reported in this paper, ovine strains of *B. chauvoei* from Montana sheep blackleg outbreaks and recognized bovine strains of *B. chauvoei* were indistinguishable in cultural, biochemical, guinea-pig protection and cross-agglutination tests.

Author's Summary.

Microbic Dissociation of B. Subtilis. M. H. Soule, J. Infect. Dis. **42**:93, 1928.

Pure cultures of *Bacillus subtilis*, old laboratory strains, as well as strains recently isolated from air, milk, water and hay infusions, yield at least three types of colonies. The S, or normal type, is characterized by snakelike motility, granular growth in broth, "ray crown" colonies on gelatin and smooth compact regular colonies on agar, which on continued incubation develop a halo of R type growth, with secondary colonies or lytic areas. The R, or resistant, type grows in long filaments, is nonmotile and produces on agar flatly convex colonies, stippled, yellowish, dry, and frosted — typical anthrax-like Medusa head colonies. The P, or phantom, type appears to be intermediate to S and R, with a tendency to form R colonies, and on agar grows in small, flat, nearly invisible colonies made up of motile diplobacilli. All types are gram-negative. The effects of homologous and nonhomologous serums, of rapid transferring, of heat and of different tensions of oxygen and carbon dioxide on dissociation, and the antigenic relationship of the S and the R types, are described.

From Author's Summary.

Bacterium Melaninogenicum from Normal and Pathologic Tissues. Kenneth L. Burdon, J. Infect. Dis. **42**:161, 1928.

Bacterium melaninogenicum constantly inhabits healthy mucous membranes and takes a prominent part in various pathologic processes.

Author's Summary.

Quantitative Estimation of Casein Hydrolysis by Cl. Botulinum. Gail M. Dack and Willard L. Wood, J. Infect. Dis. **42**:172, 1928.

Clostridium botulinum will grow and produce toxin in a medium containing casein and peptone. The peptone is first utilized for growth; after a secondary incubation period the casein is broken down and used in further growth and toxin production. The curve for the toxin titer shows a relationship to casein hydrolysis as indicated by the total nitrogen curve.

Authors' Summary.

Toxin Production and Proteolytic Activity of Clostridium Botulinum in Peptone and Peptone Egg White Medium. Gail M. Dack, Willard L. Wood and Sophie A. Dehler, J. Infect. Dis. **42**:176, 1928.

The growth curve of *Clostridium botulinum*, type A, was found to reach a maximum at the same time as the curve for the toxin titration, both in plain peptone broth and in a medium of coagulated egg white with peptone.

Authors' Summary.

THE ATTENUATION AND TOXIN PRODUCTION OF THE DIPHTHERIA BACILLUS. AUGUSTUS WADSWORTH and MARY W. WHEELER, J. Infect. Dis. **42**:179, 1928.

In the synthetic medium of Uschinsky, attenuation of the diphtheria bacillus was obtained in one instance with complete loss of virulence and toxin production. Neither virulence nor toxin production could be restored by cultivation in mediums or by animal passage.

In a synthetic peptone medium, free from meat infusion, containing only the chlorides, sulphate and phosphates of sodium, calcium and magnesium, with dextrose as a source of energy and peptone as a source of nitrogen, the diphtheria bacillus not only grew but produced toxin. A potent toxin, however, was obtained only when in the preparation of this medium the calcium and phosphate ions were heated together in the presence of the peptone. The calcium ions could be replaced by equivalent amounts of barium or strontium but not by magnesium or manganese. The addition of a small amount of colloidal calcium phosphate to an infusion-free peptone medium, which, without the colloidal calcium phosphate was not favorable for toxin production, increased toxin production from tenfold to one hundred-fold. The precipitation of calcium, however, from toxin, after it had once been produced by the diphtheria bacillus, did not alter the potency of that toxin.

AUTHORS' SUMMARY.

A KERATITOGENOUS VIRUS OF PULMONARY ORIGIN. J. R. PERDRAU, J. Path. & Bact. **31**:9, 1928.

The virus of herpes has not been recovered once in the course of an examination of fifty lungs from human beings, twenty-six of which were frankly pneumonic. A keratitogenous "virus" is present in lungs which are the site of a pathologic lesion varying from simple congestion and fibrinous exudation to true pneumonia. By cross immunity experiments this virus is found to be unrelated to that of herpes. An attack of this particular form of keratitis leaves the cornea more sensitive to a second and third attack.

AUTHOR'S SUMMARY.

SUPPURATIVE ARTHRITIS DUE TO HEMOPHILIC BACTERIA. J. F. TAYLOR, Lancet **1**:1341, 1927.

In three cases of suppurative arthritis in young persons bacteria of the type of *Bacillus influenzae* were recovered in pure culture. Only a few similar cases have been recorded.

THE "MALADIE DE MARSEILLE" (EXANTHEMATIC FEVER OF UNDETERMINED ORIGIN); IS IT THE SAME DISEASE AS TYPHUS FEVER? E. BURNET and D. OLMER, Arch. Inst. Pasteur de Tunis **16**:317, 1927.

Pointing out that tsutsugamushi of Japan, Rocky Mountain spotted fever and tabardillo of Mexico all appear to be true forms of Brill's disease, the authors present preliminary experimental studies concerning the possible grouping of the "maladie de Marseille" with these diseases. An outline for studying the possible correlation includes: clinical — special observation of certain specified cardinal features; epidemiologic — study of possible carriers, as lice, rodents and various insects, or of the association of the disease with professions (grain workers et al.); Weil-Felix reaction — checking the serum of the patients, beginning with the fourteenth day of the disease (fever), and special research with the various strains of Proteus X19 used, including studies of serums collected from other febrile diseases; experimental inoculations — employing first guinea-pigs and monkeys, as they are known to respond, with research on other animals, use of cerebral material, observation of guinea-pigs up to thirty days, special care in temperature charting, following to the "2nd degree," i. e., inoculating secondary animals from the primary ones, if these do not show fever, to avoid missing the "unapparent"

form or type in the primary animals; immunity reactions — testing cured pigs with a known virus to ascertain immunity after from four to eight weeks. The authors consider the matter still open, but their experimental work does not greatly favor the identification of the Marseille disease with typhus.

M. S. MARSHALL.

CERTAIN RULES TO FOLLOW IN EXPERIMENTAL WORK WITH SPIROCHETES. CHARLES NICOLLE and CHARLES ANDERSON, Arch. d. Inst. Pasteur de Tunis **16**:333, 1927.

The authors present their rules under the following headings: (*a*) avoiding the introduction of spirochetes from outside; (*b*) avoiding contamination with a similar type simultaneously kept in the same laboratory; (*c*) control of purity; (*d*) assuring successful passages; (*e*) avoiding infection on the part of the experimenter.

M. S. MARSHALL.

TRACHOMA AND GRANULAR CONJUNCTIVITIS. CHARLES NICOLLE, Arch. Inst. Pasteur de Tunis **16**:378, 1927.

This review, originally appearing in Russian (Arch. d'opht., 1927, vol. 3) discusses the present status of the knowledge of trachoma, and the difficulties in experimental investigation.

M. S. MARSHALL.

RELATION OF CERTAIN HUMAN GRANULAR CONJUNCTIVITIS TO THE NATURAL GRANULAR CONJUNCTIVITIS OF THE RABBIT. UGO LUMBROSO, Arch. Inst. Pasteur de Tunis **16**:385, 1927.

Discussing the various types of granular conjunctivitis, the author emphasizes the possibility that these diseases are soil-borne, and hopes that increased application of hygiene will in time eliminate them.

M. S. MARSHALL.

TRACHOMA IN TUNIS. M. ZACHERT, Arch. Inst. Pasteur de Tunis **16**:391, 1927.

The infection of children in Tunis with trachoma is always precocious (first months or years after birth); its evolution is rapid and serious (frequent scarring). The infection of children in Poland is late (age of infected children from 10 to 15 years), and its development is slow and benign, without complications. The infectious agent of trachoma is much more virulent in Tunis than in Poland.

AUTHOR'S CONCLUSIONS.

INFLUENCE OF PROTEIN THERAPY ON THE PARTIAL PREVENTION OF THE PARADOXIC PHENOMENON OF BEHRING. LIUGI LORENZO SCATTOLIN, Boll. d. Ist. sieroterap. milanese **6**:417, 1927.

The inoculation of small quantities of peptone every day at the same time that small doses of diphtheria and tetanus toxins are given produces an attenuation of the symptoms of intoxication. This fact is not without importance, and it may throw light on various phenomena observed in the prevention and evolution of infectious diseases.

A. J. SALLE.

THE ELIMINATION OF RABIES VIRUS BY WAY OF THE DIGESTIVE TRACT. V. PUNTONI, Rendiconti d. r. Accad. naz. d. Lincei **6**:342, 1927.

Previous investigators have concluded that the path of elimination of rabies virus is by way of the salivary and pancreatic glands only. The author shows that this is only partially true. In autopsies on dogs which died of rabies it is seen, with a certain frequence, that inflammation of the oral mucous membranes is also present. These facts were frequently noticed previously by others.

Manouelian found inclusions similar to the Negri bodies in the nerve endings of the tongue and formulated the hypothesis that the virus is eliminated by this channel. The old theory of the elimination of the virus by way of the salivary glands must now be substituted by another theory, more ample and comprehensive, which considers all of the mucosa and almost all of the glands of the digestive tube as surfaces of elimination of the rabies virus.

A. J. SALLE.

SKIN REACTIONS WITH CLIMATIC BUBO. O. FISCHER, Klin. Wchnschr. **7**:255, 1928.

The intracutaneous reaction in patients with lymphogranuloma inguinale caused by the injection of exudate obtained from a necrotic lymph gland, diluted from five to eight times with physiologic sodium chloride solution and sterilized by heating for one and one-half hours at 60 C., occurs also in patients with climatic bubo. The exudate from patients with either lesion is equally active, and such results indicate further that the two diseases are essentially identical.

AUTHOR'S SUMMARY.

THE DIFFERENTIATION OF THE TRUE PARATYPHOSUS B. FROM THE BRESLAU-ENTERITIS BACTERIA ON AMMONIUM CHLORIDE-RHAMNOSE-AGAR. K. L. PESCH and A. MASCHKE, Klin. Wchnschr. **7**:401, 1928.

Ammonium chloride as the only N constituent and rhamnose as the only carbohydrate, sustain the growth of the Breslau but not the Schottmüller and Gärtner bacteria. The growth or absence of growth on this medium after two days' incubation is a clear differentiating characteristic for these closely related bacteria.

E. F. HIRSCH.

Immunology

SPECIFIC TREATMENT OF LOBAR PNEUMONIA. RUSSEL L. CECIL, Arch. Int. Med. **41**:295, 1928.

Cecil reviews the development of specific agents against pneumonia. Significant advance followed the discovery of biologically different types of pneumococci by Neufeld and Handel in 1910. Pneumococcus infection in man and animal induces an immunity against that type of pneumococcus but not against others. The immune bodies are neutralized by a type-specific soluble substance, a polysaccharid originating in the pneumococcus capsule. Death in pneumonia is associated generally with blood infection.

Many favorable reports have been made on specific treatment of pneumonia, and some unfavorable ones. Cecil reports on the use of antipneumococci whole serum, Huntoon's pneumococcus antibody solution and Felton's concentrated antipneumococcus serum. Pneumonia in man and experimental pneumonia in monkeys was studied. With all the preparations, patients with type I pneumonia reacted well to treatment, those with type II pneumonia were favorably influenced, while those with type III infections did not receive any benefit. Good results obtained occasionally in type IV pneumonia seem to be due to foreign protein reactions. Even after invasion of the blood stream in type I and II infections, it is possible to sterilize the blood and cure the patient by specific treatment.

H. R. FISHBACK.

IMMUNITY TO TUBERCULOSIS. JOHANNES HEIMBECK, Arch. Int. Med. **41**:336, 1928.

Heimbeck reports that 48 per cent of the nursing students entering the Municipal Hospital of Oslo, Norway, in four years have given a positive Pirquet test. The Pirquet test is positive in 85 per cent of the school children of Oslo at the age of 9. Heimbeck interprets this as a burning out of infections

by the time adult age is reached and a disappearance of immunity, so that reinfection with tuberculosis may occur as in childhood. Nurses who gave a negative Pirquet reaction were rendered positive to this test by the subcutaneous injection of Calmette's avirulent tubercle bacilli. In one class, twelve were so treated and are free from tuberculosis, while of eleven students who gave a negative reaction to the Pirquet test and who remained untreated, four contracted tuberculosis.

H. R. Fishback.

An Improved and Simplified Method for Making a Standard Undenatured Tuberculin of any Desired Strength and a Chemical Assay for the Same. F. B. Seibert, J. Biol. Chem. **78**:345, 1928.

A virulent human strain of the tubercle bacillus is planted on a nonprotein-containing, synthetic medium and the culture incubated for three months. The ensuing growth is removed by Berkefeld filtration and the filtrate containing tuberculin is concentrated, after the addition of 0.5 per cent of phenol, by ultrafiltration through collodionized alundum shells. The potency of the undenatured tuberculin preparations yielded by this procedure can be approximated, in a purely chemical way, in terms of content of substance precipitable by trichloracetic acid.

Arthur Locke.

Effect of Antirheumatic Drugs on the Arthritis and Immune Body Production in Serum Disease. C. L. Derick, C. H. Hitchcock and H. F. Swift, J. Clin. Investigation **5**:427, 1928.

In thirty-four patients treated with serum, either neocinchophen or acetylsalicylic acid was given, beginning from one to two days after the last injection of serum, and continuing for from ten to fourteen days. Comparison of these cases is made with sixty-six cases from the hospital records in which the patients were previously treated with serum, as to frequency of occurrence and severity of arthritis and urticaria.

In the cases in which a drug was administered, serum tests were carried out for the presence of antigen (horse serum) and antibodies (precipitins). Formation of precipitins was restricted, rarely rising above 1 : 40, but a serum content of precipitin of 1 : 400 usually was associated with arthritis.

The course of treatment with drugs generally prevented arthritis, but was ineffective against other manifestations of serum sensitiveness. The theory is advanced that urticaria is the result of active sensitization of the skin, while arthritis follows passive sensitization of the joints by circulating antibodies in the serum.

H. R. Fishback.

The Rôle of Clasmatocytes and Connective Tissue Cells in Nonspecific Local Cutaneous Immunity to Staphylococcus. S. O. Freedlander and J. A. Toomey, J. Exper. Med. **47**:663, 1928.

Plain broth is just as effective as specific broth filtrate if used as a skin compress for the protection of guinea-pigs against a subcutaneous injection of *Staphylococcus aureus*. Plain broth compresses applied for forty-eight hours previous to bacterial injection sometimes prevent the death of the animal and practically always alter the inflammatory reactions. This protection is not specific and is localized to the area "compressed." The protection lasts at least twenty-four hours after the removal of the compress. Broth compresses applied to the abdominal wall of a guinea-pig for forty-eight hours produced definite histologic changes, especially in the subcutis, i. e., edema, proliferation of clasmatocytes, thickening of the epidermis together with a moderate exudation of polymorphonuclears and small mononuclear cells. The histologic response to the subcutaneous injection of staphylococci was different in the control and in the prepared animal with broth. In the animal prepared with broth, there was an increase in clasmatocytes, and fibroblasts with a dense exudation of polymorphonuclears, which latter,

in the main, did not degenerate. The clasmatocytes phagocyted bacteria early and later engulfed the polymorphonuclears, while the fibroblasts rapidly walled off the lesion. The result was a localized abscess which either came to the surface and ruptured, or was absorbed and organized.

AUTHORS' SUMMARY.

IMMUNOLOGICAL STUDIES IN RELATION TO THE SUPRARENAL GLAND. J. MARMORSTON-GOTTESMAN and DAVID PERLA, J. Exper. Med. **47**:713 and 723, 1928.

After having studied the formation of hemolysin in normal rats under standard conditions, the formation was studied in rats from which the suprarenals had been removed with the following results: Bilateral suprarenalectomy in rats subsequently injected intraperitoneally with 1 cc. of a 10 per cent suspension of sheep cells resulted in a depression of hemolysin titer during five weeks following the operation, the depression being most marked during the first week. Bilaterally suprarenalectomized rats injected intraperitoneally two weeks after operation with 1 cc. of undiluted sheep cells gave hemolysin titers higher than did normal rats. The quantity of antigen necessary to yield the maximum titer in suprarenalectomized rats two weeks after operation is ten times the quantity necessary to yield the same titer in normal rats. Traumatization of the perisuprarenal tissue in rats produced the same effect on the antibody-forming capacity as suprarenalectomy.

ON INDIVIDUAL DIFFERENCES IN HUMAN BLOOD. K. LANDSTEINER and PHILIP LEVINE, J. Exper. Med. **47**:757, 1928.

A clearcut differentiation of human blood, aside from the blood groups, could be made by means of special agglutinating immune serums. The observations point to the existence of several agglutinable factors for which no agglutinins are demonstrable in normal human serums. In view of the latter circumstance, the results reported do not imply any change in the scheme of the four blood groups. The body of serologic evidence leads to the inference of a high degree of biochemical differentiation among individuals.

AUTHORS' SUMMARY.

SPECIFIC HYPERSENSITIVENESS. ELLA F. GROVE, J. Immunol. **15**:3, 1928.

Efforts to sensitize passively the skin of the chimpanzee and lower monkeys did not give positive results. If confirmed, these results would mean that animals most nearly related to man behave more like the rabbit and the guinea-pig in lacking certain elements that appear to be characteristic of man.

HEREDITY IN SPECIFIC HYPERSENSITIVENESS. J. A. CLARKE, JR., H. H. DONNALLY and A. F. COCA, J. Immunol. **15**:9, 1928.

Evidence is presented indicating that while hay-fever and bronchial asthma may be closely associated, there is a tendency to independent transmission which would suggest that the bronchial tissues are subject to hereditary influences that differ from those of the upper respiratory tract.

THE INFLUENCE OF HEPARIN ON ANAPHYLACTIC SHOCK IN THE GUINEA-PIG. F. RENE VAN DE CARR and O. B. WILLIAMS, J. Immunol. **15**:13, 1928.

Heparin injected into the circulation in quantities sufficient to prevent clotting for from twenty-four to forty-eight hours or longer may reduce or prevent anaphylactic shock and other anaphylactic symptoms in the sensitized guinea-pig. Heparin may delay, but does not prevent, death from peptone shock.

THE EFFECT OF FORMALDEHYDE ON SPECIFIC COMPLEMENT FIXATION SYSTEMS. C. E. REYNER, J. Immunol. **15**:37, 1928.

The addition of formaldehdye in suitable concentration increases the amount of complement fixed by a specific fixation system. The addition of formaldehyde

to a specific system will reveal antibody formation more promptly during immunization than will the usual method.

AUTHOR'S SUMMARY.

THE EFFECT OF BACTERIOPHAGE ON LEUKOCYTOSIS AND PHAGOCYTOSIS. A. R. NELSON, J. Immunol. **15**:43, 1928.

The intravenous injection of antistaphylococcus bacteriophage exerts a marked influence on the type of leukocytic response elicited by susceptible staphylococci, but has only a slight effect on the type of response due to resistant organisms. This influence is characterized by the fact that a single injection of the bacteriophage together with susceptible organisms causes a decrease in the degree and time of appearance of the leukocytosis as compared with that due to susceptible organisms alone. Repeated injections of the bacteriophage increase the period of leukopenia and decrease the degree of luekocytosis as compared with that due to susceptible organisms alone. The bacteriophage does not alter the type of response due to resistant organisms in any way comparable to the effect of the bacteriophage on the type of leukocytic response due to susceptible organisms. When leukocytes and organisms are brought into contact with the antistaphylococcus bacteriophage in vitro, in either the presence or absence of serum, the degree of phagocytosis in the case of susceptible organisms is markedly increased, whereas in the case of resistant staphylococci the degree of phagocytosis is practically unaffected. When antistaphylococcus bacteriophage is injected intravenously, there is an almost immediate increase in the phagocytic power of the leukocytes for susceptible organisms, whereas for resistant organisms the phagocytic power remains practically unaltered. The opsonic power is not altered appreciably in either case.

AUTHOR'S SUMMARY.

ANAPHYLAXIS. NOBLE B. SHERWOOD and C. M. DOWNS, J. Immunol. **15**:65, 73 and 77, 1928.

Allergic response was obtained from the embryonic chick heart before connection of the heart with the central nervous system had been established. Allergic response was obtained also in turtles under certain conditions, and Downs was able to sensitize turtles actively by means of mammalian serum. This specific response resembles vagus stimulation of the turtle's heart.

INTRADERMAL TESTS AS AN AID IN THE DIAGNOSIS OF PARASITIC INFESTATION. MATTHEW BRUNNER, J. Immunol. **15**:83, 1928.

Dermal testing with extracts of parasites results in reactions which have been proved to be immunologically specific, and are indicative of present or past infestation; it is therefore a valuable aid in the clinical diagnosis of helminthiasis.

AUTHOR'S SUMMARY.

A PRECIPITIN TEST IN EXPERIMENTAL TRICHINIASIS. GEORGE W. BACHMAN, J. Prev. Med. **2**:35, 1928.

Satisfactory antigen solutions of *Trichinella spiralis* were prepared by freeing the larvae from infected meat by artificial digestion, and by hydrolyzing the dried trichinella powder in 0.1 per cent hydrochloric acid. This acid solution can be used unmodified for artificial immunization of rabbits, but as test antigen in vitro precipitin tests, it must be neutralized with 1 per cent sodium hydroxide and used at a p_H of 7.2 to 7.4. In artificially immunized rabbits, precipitins were detectable in high concentration five days after the last injection of the antigen, remaining in a high concentration (demonstrable with antigen dilutions of 1:2,000 in terms of dry weight of the trichinella powder) until about the fortieth day; then they gradually disappeared and were not demonstrable after about the seventieth day. In rabbits infected with *T. spiralis* by being fed on trichinous meat, precipitins were not demonstrable until the thirtieth day; then the titer

increased rapidly (demonstrable with antigen dilution of 1 : 3,500 in terms of dry weight of the trichinella powder) until the ninetieth day and were still detectable in two cases after 227 days and in one case after 367 days (end of observation period). Precipitins were demonstrable in the serum of a man infected with *T. spiralis* in an antigen dilution of 1 : 3,500.

AUTHOR'S SUMMARY.

HYDATID FLUID AS AN ANAPHYLACTIC ANTIGEN. C. H. KELLAWAY, J. Path. & Bact. **31**:141, 1928.

A method is described by which bicarbonate Ringer's solution which is being oxygenated may be kept at constant p_H with a constant partial pressure of carbon dioxide. The pressure of host serum protein in most samples of hydatid fluid is shown, in confirmation of Graetz and others, by the use of the isolated uterus of the guinea-pig. Host serum protein is not necessarily a constituent of the fluid in the living parasite for it is virtually impossible to remove fluid from within a mother cyst without some of it coming in contact with the adventitia and in in vitro experiments mother and daughter cyst though permeable to dyes of low molecular weight are not readily permeable to oxyhemoglobin or to serum proteins. In addition to host serum proteins, another anaphylactic antigen is present in hydatid fluid. This is demonstrated by using concentrated hydatid fluid of several species. It does not pass through the filters described and may therefore be concentrated by ultrafiltration.

AUTHOR'S SUMMARY.

EXPERIMENTS UPON THE RELATIONSHIP OF COMPLEMENT FIXATION TO PRECIPITATION. NEIL E. GOLDSWORTHY, J. Path. & Bact. **31**:220, 1928.

Precipitation is a process which may be divided into three stages: (1) the phase which follows the mixing of the antigen and antibody, during which the mixture remains clear; (2) the phase of opalescence and turbidity, which merges insensibly into the preceding and succeeding phases, and (3) the phase of particulation or precipitation in its narrower sense. Complement fixation occurs during the early phases of the precipitation reaction, and the time necessary to produce these phases depends on the proportions in which antigen and antibody are present in the mixture, the potency of the antiserum. Both precipitation and complement fixation depend on these two factors. A mixture of antigen and antiserum containing the optimal proportions for rapid particulation quickly reaches the peak of its complement-binding capacity and is soon beyond that point, after which the amount of complement fixed is practically nil. Mixtures where either antigen or antibody is present in relative excess are slower to attain their maximal fixing capacity. Mixtures where antigen is but moderately in excess retain their opalescence and complement-fixing powers in a remarkable manner. In a series of mixtures containing antigen and antibody in various proportions, maximal fixation moves from point to point in the series in a perfectly orderly way as each mixture reaches that phase of precipitation most favorable for fixation. The greater the departure from optimal proportions the slower is the attainment of this maximal fixing capacity. Complement fixation is a function of the size of the "particle" of a precipitate.

AUTHOR'S SUMMARY.

A PRECIPITINOGEN OBTAINED FROM CULTURES OF B. AERTRYCKE MUTTON (SALMONELLA AERTRYCKE). FRANK CHARLES HAPPOLD, J. Path. & Bact. **31**:237, 1928.

A precipitinogen has been prepared from broth cultures of *B. aertrycke Mutton*. It appears to be identical with the antigen which stimulates the production of agglutinins to a heat-stable antigenic form of the organism. Preparations which react in vitro always stimulate the production of antibodies in vivo, but a solution may be actively antigenic in the rabbit and fail to produce a precipitate in vitro. Processes which would be calculated to modify or reduce proteins have effected a destruction of the antigen and other evidence of the protein nature of this substance is presented.

AUTHOR'S SUMMARY.

COMPARATIVE STUDY OF CULTURES OF TISSUES INOCULATED EITHER WITH BOVINE TUBERCLE BACILLI OR WITH THE BCG TUBERCLE BACILLUS. ALEXANDRE MAXIMOW, Ann. de l'Inst. Pasteur **42**:225, 1928.

The first rôle of defense is a specific reaction on the part of the epithelioid cells and giant cells. The epithelioid cells phagocytize the tubercle bacilli, surrounding their colonies, and uniting in groups to become confluent as giant cells, forming the tubercle. In tissue cultures, the epithelioid cells of the rabbit are powerless in combating the bovine strain, apparently through a toxic action on the phagocytes, manifested even over some distance. On the other hand, the BCG bacilli do not show, in vitro, any definite action toxic to the cellular elements; even the sensitive lymphocytes may live and multiply in the immediate proximity of the bacilli. However, the BCG bacilli continue to multiply, the polyblasts being insufficient to overcome them. Absorbed bacilli seem to have no unfavorable action on the phagocytes, whereas phagocytes ingesting bovine bacilli always degenerate. Fibroblasts show a considerable resistance to the BCG bacilli. The bacilli did not vary during the period observed in the weakness of its virulence, always producing a general tuberculous infection in guinea-pigs following subcutaneous injection.

FROM THE AUTHOR'S CONCLUSIONS.

EXPERIMENTS WITH BCG VACCINE. REPORT OF THE UKRANIAN COMMISSION, Ann. de l'Inst. Pasteur **42**:246, 1928.

BCG vaccine produced only local lesions, with no tendency to become virulent, in guinea-pigs weakened by various procedures or inoculated repeatedly with tuberculin. Cows were successfully vaccinated, organisms from the milk being found avirulent. Evidence thus far presented by the commission strongly favors the vaccination procedure.

M. S. MARSHALL.

REPORT OF THE INTERNATIONAL CONFERENCE ON RABIES, PARIS, APRIL, 1927. Ann. de l'Inst. Pasteur, Supplement issued with vol. 42, 1928.

It is impossible to abstract the specific information contained in the 171 pages of this report. The subjects considered are: (1) the nature of the virus of rabies (A. C. MARIE); (2) technic of the vaccination of man, with its various modifications (A. C. MARIE); (3) paralysis consequent to antirabies treatment (P. REMLINGER); (4) local accidents from antirabies treatment (P. REMLINGER); (5) antirabies vaccination of animals (H. VALLÉE). The status of various phases of rabies investigations and practices are summarized and commented on, and many details in technic are described in some detail.

M. S. MARSHALL.

PRODUCTION OF ANTIBODIES IN INFANTS. M. SCHTEINGART and R. R. CERVINI, Semana méd. **1**:415, 1928.

In eleven infants, aged from 41 days to 13 months, inoculation of typhoid vaccine gave rise to formation of agglutinins and other antibodies.

IMMUNITY IN RECURRENT FEVER. E. S. HERONIMUS, Centralbl. f. Bakteriol. **105**:394, 1928.

Heronimus confirms the observations of other workers that a reinfection of immune mice with recurrent spirochetes often leads to a subsequent infection of the brain. Humoral immunity in relapsing fever is relatively weak and labile, the immunity having the character of an "infection immunity."

PAUL R. CANNON.

THE EFFECT OF A TUBERCULOUS INFECTION ON THE FORMATION OF HEMOLYSINS. H. DOLD and H. GROSS, Centralbl. f. Bakteriol. **104**:343, 1927.

The infection of twenty rabbits with tuberculosis from one and one-half to three months before starting injections of sheep's corpuscles had no influence on

the formation of hemolysin, the titer being as high as with a similar group of normal controls. The authors suggest that the difference in their results as compared with those of other workers may be due to the fact that guinea-pigs were used by others in which generalized organ tuberculosis was obtained whereas in the experiments of Dold and Gross, only a lymphatic tuberculosis was present.

PAUL R. CANNON.

THE INFLUENCE OF SENSITIZATION WITH LENS PROTEIN ON THE FORMATION OF TRAUMATIC CATARACT IN RABBITS. WILLY BENDER, Centralbl. f. Bakteriol. **106**:7, 1928.

The sensitization of rabbits by the subcutaneous and intraperitoneal injection of swine lens protein, followed by the injection into the lens of 5 per cent saline, according to the method of Selenkowsky, leads to a more marked and more rapidly developing cataract than is the case in unsensitized animals.

PAUL R. CANNON.

RELATION OF EOSINOPHILIA AND IMMUNE BODY FORMATION. K. HAJÓS, Ztschr. f. d. ges. exper. Med. **59**:389, 1928.

The author gives a curve to show a proportional increase of eosinophils and agglutinins in a dog injected with typhoid bacilli. The eosinophilia of certain infections appear to run parallel to the formation of immune substances. Absence of eosinophils during immunization is regarded as a sign of diminished response.

B. LUCKE.

ISOAGGLUTINATIONSSTUDIEN. O. SIEVERS, Acta path. et microbiol. Scandinav. **4**:285, 1927.

In connection with a series of blood group determinations in Finnland, previously reported (Bidrag till kännedom of Finlands Natur och Folk, 1927), Sievers studied the inheritance of the blood groups in 286 families. The results of this study are reported in the present paper. The proportions of the children to be expected in each group from each type of mating are calculated, based on the observed frequencies of the hereditary factors. The percentages found by the author agree closely with those to be expected on the basis of the multiple allelomorph theory, and are not in agreement with those to be expected based on the theory of independent pairs of factors.

Similar comparisons between expected and observed results are made, using the summarized figures of family studies of twenty-four authors. The results favor the multiple allelomorph hypothesis, although the agreement is not as close as in the case of the author's own figures. When only the results obtained by workers since 1926 are used, the agreement is much closer, owing, according to the author, to the fewer mistakes in technic made in the later works.

A short discussion of the relation of blood groups to pathologic conditions is presented. Percentages of the four groups are given for persons suffering from syphilis, nervous disorders, heart diseases, cancer and other diseases. Signification deviation from the expected figures for normal persons is not found in any of the pathologic conditions studied.

L. H. SNYDER.

PASSIVE TRANSFER OF IDIOSYNCRASY. K. H. BAAGÖE, Act. Path. et Microbiol. Scandinav. **4**:302, 1927.

In seven cases of asthma, the effort to transfer the specific sensitiveness to animals by means of the serum of the patient succeeded in only one case (fish). In six of fourteen cases, the author succeeded in making the skin of healthy persons specifically sensitive by injecting the serum of the patient.

RASSENBIOLOGISCHE UNTERSUCHUNGEN AUF GRÖNLAND. E. BAY-SCHMITH, Acta path. et microbiol. Scandinav. **4**:310, 1927.

The blood groups of Greenland Eskimos are found to be in the following proportions: group 0, 39 per cent; A, 55 per cent; B, 4 per cent; AB, 1 per cent. Increasing contact with the outside world appears to increase the per cent of group 0 and decrease that of group A. Curves are presented showing the trend of increase of 0 and decrease of A from Eskimos through Greenlanders to Indians. Studies are also presented on the sedimentation rates in the four groups in different regions. A study of the blood groups in disease shows an increased proportion of group A in infectious diseases.

L. H. SNYDER.

THE ANATOMIC CHANGES AFTER INTRACAROTID SERUM INJECTION INTO THE BRAIN IN GUINEA-PIGS. SVEN INGVAR, Acta Path. et microbiol. Scandinav. **4**:349, 1927.

Extensive capillary hemorrhages developed in the central nervous system, especially in the medulla owing to injuries to the capillary endothelium. These changes explain satisfactorily the symptoms observed in the animals in which injections have been made.

Tumors

SPONTANEOUS DECIDUOMATA IN PSEUDOPREGNANCY WITH LOW VITAMIN E. H. M. EVANS, Am. J. Physiol. **85**:149, 1928.

In rats in which a false reaction of pregnancy had been induced by copulation with a vasectomized male, there was occasionally found the development of tumors of maternal placental tissue, similar to those induced by Loeb by injury to the endometrium in the guinea-pig. This development was greatly influenced by a deficiency of vitamin E in the diet, since it occurred in 60 per cent of such animals, as compared with 4 per cent of those on normal diet. That the reaction was not caused by the descent of an unfertilized ovum was shown by its fairly regular occurrence in animals in which the tip of the uterine horn was occluded by excision. The tumors were formed from the connective tissue of the endometrium, and resembled the placenta of the twelfth day of pregnancy, minus the fetal elements.

H. E. EGGERS.

METASTASES IN THE BONES IN PRIMARY CARCINOMA OF THE LUNG: A REVIEW OF SO-CALLED ENDOTHELIOMAS OF THE BONES. E. F. HIRSCH and E. W. RYERSON, Arch. Surg. **16**:1, 1928.

This is a valuable critical analysis of the "so-called endotheliomas of the bones." The authors have reviewed the literature, and show that the diagnosis of endotheliomas has often been made on incomplete evidence. They point out the infrequency of complete postmortem investigations for primary carcinoma. In their own experience they have two cases in which the tissue removed from the bones was diagnosed endothelioma, whereas postmortem examination revealed the presence of what they considered primary carcinoma of the lungs. In one of their cases a diagnosis of secondary carcinoma of the bones was made, but a careful physical examination failed to reveal a primary growth. The patient, a boy, aged 6, died a year later of carcinoma of the lungs. The diagnosis of tumors of the bones from their microscopic appearance, is, as they point out, a hazard.

N. ENZER.

ACTION OF HIGH FREQUENCY CURRENTS UPON TRANSPLANTABLE MOUSE SARCOMA. J. W. SCHERESCHEWSKY, Pub. Health Rep. **43**:927, 1928.

The conclusions seems justified that by exposing transplantable tumors of two strains (mouse sarcoma C. R. 180 and the Rous fowl sarcoma) to the action of an intense electrostatic field excited by high-frequency oscillations of from 68,000,000

to 66,000,000 cycles per second, it is possible in a sufficient number of instances to be significant, to produce complete recession of the tumor and consequent recovery of the tumor-bearing animal.

The method, in its present state of development, has obvious limitations in that it is confined to the treatment of subcutaneous growths which can readily be included between the plates of the treatment electrodes. Within these limitations, however, the action of the electrostatic field proved highly inimical to tumor growth and development, only twenty-two, or 5.5 per cent, of 400 mice experimented with actually dying of tumor. With mice, the problem was not so much the destruction of the tumor as to preserve the mouse free from intercurrent infections until complete recession and solid recovery had taken place.

The impression was derived that mice which had undergone treatment were, for a time at least, more susceptible than normal mice to certain bacterial infections, which brought about many more deaths than did the tumors. The treatment, too, when a certain dosage was exceeded, was able of itself to cause death. Also the lack of experience as to correct dosage, proper insulation of electrodes and similar factors was responsible for a considerable mortality which in future experiments it should be possible to avoid.

Since stable and efficient apparatus for generating these high-frequency currents has been available to the laboratorian only for about four years or so, the action of these currents on living tissues has been but little investigated. Certainly, no previous data of practical value as to physiologic action were available for guidance in the experiments here reported.

Even now we are evidently only on the threshold of the possibilities for investigation. Much remains for study, particularly with respect to the changes wrought in living cells by the application, in this particular way, of these currents. Studies along these lines are now under way and will be made the subject of future report.

The hypothesis that the frequency at which these currents are produced may have the specific quality of attacking certain cells more than others is interesting and worthy of future experimentation. Observations already collected suggest that this may be the case. The first paper published on the action of those currents, to which reference has been given, shows plainly that their action at all frequencies is not the same but that pronounced differences exist.

In a small series of experiments a much higher frequency (135,000,000 cycles per second) than the one usually employed proved to be without particular effect on the tumor cells of the mouse sarcoma, while a preliminary study of sections of treated tumors removed immediately after exposure shows that normal tissue cells surrounding the tumor seem to be less attacked by the high-frequency currents than the tumor cells themselves.

So far as the possible therapeutic application of this method and these currents to human disease is concerned, a considerable period of observation and investigation is required before one would be justified in making such attempts, although one may hope that the results of animal experimentation foreshadow, albeit though dimly at present, results which may well be of practical utility.

Finally, it may be said that the results herewith reported distinctly encourage further investigation and study. The hope is expressed, too, that others will investigate this field with its many seeming possibilities and thereby increase the likelihood of recording observations which may be susceptible of practical application.

AUTHOR'S SUMMARY.

BLOOD CHANGES OCCURRING DURING THE COURSE OF TREATMENT OF MALIGNANT DISEASE BY LEAD, WITH SPECIAL REFERENCE TO PUNCTATE BASOPHILIA AND THE PLATELETS. RONALD WINSTON BROOKFIELD, J. Path. & Bact. **31**:277, 1928.

A considerable temporary degree of anemia is produced by the injections. This is due to peripheral destruction of red blood cells by the lead which exerts its

maximum effect immediately after an injection, while it is still circulating in the blood stream. The tendency of the red count to return rapidly to normal is due to an increased activity of the bone marrow as shown by the large number of reticulated cells which appear soon after the commencement of the injections. The origin of stippled red cells has been investigated. By a large series of comparative counts, during the progress of treatment, these were found to be closely related to the reticulated cells and in such a manner as to justify the conclusion that stippled cells are young cells altered by a degenerative process. This conclusion was confirmed by morphologic experiments with different dilutions of cresyl blue by the use of which all transitions between typical reticulum and typical stippling could be produced. Some evidence has been brought forward to confirm the view that stippling is not present as such while the blood cells are circulating, but appears after the blood has been shed. No change definitely attributable to lead has been found in the white cells. The effect of two different preparations of colloidal lead on the platelets has been studied. Injections of a suspensoid of metallic lead, lead hydroxide and lead carbonate were found to produce an immediate rise in the number of the circulating platelets, while a lead selenium compound produced an immediate fall. A definite cause could not be assigned to these differing phenomena.

AUTHOR'S SUMMARY.

ON THE METHODS OF KOTZANEFF AND OF BITTMANN FOR THE PRODUCTION OF TAR CANCER. T. ANARDI, Tumori **14**:99, 1928.

The author could not confirm the claim of Kotzaneff that rapid experimental cancer production in animals could be obtained with tar treated by an electrolytic process, nor that of Bittmann that the production of cancers of the skin by tar is facilitated by previously removing the grease which covers the skin by means of petroleum ether.

MALIGNANCY AND MALIGNANT TRANSFORMATION OF MYOMA. ANTONIO COSTA, Tumori **14**:115, 1928.

Costa describes a case of malignant myoma of the prostate in a boy, aged 5, and gives a gross picture of the tumor and several drawings of microscopic sections.

ADULT TERATOMA OF THE PLACENTA. J. KÜSTER, Arch. f. Gynäk. **133**:93, 1928.

In the placenta of a primipara, aged 33, a teratoma, the size of a walnut, was found attached to the chorion about 3 cm. from the insertion of the cord. The growth contained mature ectodermal and mesodermal tissue (bidermoma). It is suggested that the growth developed from detached somatic blastomeres.

AN ENDOTHELIAL TUMOR OF THE UTERUS. F. KLEE, Arch. f. Gynäk. **133**:186, 1928.

A tumor consisting of myomatous and endothelial elements, the latter predominating, is described. It is believed that it concerned two different tumors of independent origin.

CULTURE OF CARCINOMA CELLS IN VITRO. A. FISCHER, F. DEMUTH, H. LASER and H. MEYER, München. Med. Wchnschr. **75**:651, 1928.

Pieces of a mouse inoculation tumor were cultivated in vitro over one year. From about 120 to 130 passages were made. The culture grows in membranes that are typical for epithelial cells, without liquefying the membranes. The carcinoma tissue kept its malignancy as long as cells remained alive. The carcinoma cells were able to build up their cytoplasm from completely heterologous material and to go on living in a heterologous medium without embryonal extract

or proteoses; in fact, they proved as sensitive as leukocytes to an excess of these substances. The carcinoma tissue was able to construct its cell substance entirely out of the constituents of plasma; the cells grew faster in vitro than other tissue varieties, including leukocytes, even in a medium which was optimal for these tissues. Carcinoma tissue in vitro was more sensitive to acidification than normal tissue, and at p_H 6 it ceased to grow. It was also more sensitive than normal tissue to lowering of the oxygen tension. It grew relatively well with slight increase in the oxygen tension, but perished more rapidly than normal cells if the oxygen tension was much increased.

CHANGES IN THE SKIN AND ORGANS IN WHITE MICE AFTER PAINTING WITH TAR. GUSTAV GULDBERG, Acta Path. et Microbiol. Scandinav. **4**:276, 1927.

In the course of experiments on twenty-five mice, one developed papilloma, three incipient carcinoma and seventeen fully developed carcinoma with metastases in the lymph glands and lungs in six cases. Four of these seventeen carcinomas were composed of splindle-shaped epithelial cells, the remaining being typical squamous cell carcinoma, many of them, however, showing also less differentiated cell forms. The tar produced also degenerative and other changes in important internal organs.

Medicolegal Pathology

THE DISTRIBUTION OF BORIC ACID IN HUMAN ORGANS IN SIX DEATHS DUE TO BORIC ACID POISONING. W. D. MCNALLY and C. A. RUST, J. A. M. A. **90**:382, 1928.

Six infants, weighing approximately 3.2 kilograms each, received from 60 to 150 cc. of a saturated solution of boric acid, mistaken for distilled water. This means that each infant received about from 3 to 6 Gm. of boric acid. The amount of boric acid in the various organs of the infants was determined. The largest amounts were found in the brain and liver. The materials were ashed in an electric furnace at low red heat in the presence of an excess of sodium hydroxide solution. The boric acid was assayed according to the method in Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Washington, 1925.

CONVULSIONS DURING SURGICAL ANAESTHESIA. K. P. PINSON, Brit. M. J. **1**:956, 1927.

During nine years about 11,000 patients were anesthetized and 14 had epileptiform convulsions beginning with jerky breathing, twitching of the eyelids or arms and spreading quickly over the entire body. Ether anesthesia was used in every case. The patients were young, and in all but one there was some acute infection which had caused fever. There was apparent recovery once, but death came on unexpectedly four hours later. There were six deaths and two postmortem examinations which failed to disclose the cause of death.

Pinson believes that the convulsions are due to poor respirations and an accumulation of carbon dioxide in the blood inconsistent with life. Some support for this view is afforded by the treatment, for prompt exposure of the face of the patient to fresh air and the administration of oxygen have proved efficacious.

E. R. LE COUNT.

FATAL ACUTE PULMONARY EDEMA. M. M. POSEL, Brit. M. J. **1**:511, 1927.

The death of a factory girl, aged 16, is reported. A straw-colored froth poured from her mouth and nostrils for several hours before death, and extreme thirst was a symptom. A large fatty heart with mitral stenosis was found.

E. R. LE COUNT.

MEDICOLEGAL ASCARIDOSIS. P. DOVOLLE, Ann. de méd. lég. **8**:85, 1928.

In Tongking, 315 ascarides (some of the smallest being omitted) were found in the stomach and bowel of the body of a native girl, aged 10 years, whose parents had buried the body secretly. Five of the worms were observed in putrid fluid about the cecum in the front of which were three perforations.

E. R. LE COUNT.

STAB WOUND OF THE HEART NOT IMMEDIATELY FATAL. V. BALTHARAND, Ann. de méd. lég. **8**:117, 1928.

An Italian laborer lived sixteen hours after he incurred a self-inflicted stab wound, 1 cm. long outside and 2 mm. inside, situated 6 cm. from the apex of the heart. In the pericardial sac, there was 100 cc. of blood; in the left pleural cavity, 200 cc. The wound was closed inside by a clot.

E. R. LE COUNT.

DEATH FROM ARTIFICIAL PNEUMOTHORAX. L. CROZIER, Rev. de la tuberc. **8**:477, 1927.

Pleural reflex and air embolism are two explanations offered for the death of persons while they are undergoing artificial pneumothorax. Crozier discards the first as altogether untenable. From experiments with the injection of tincture of iodine into the pleural cavities, lungs and carotid arteries of rabbits and the injection of air into the carotid arteries, he was unable to obtain any support for the so-called pleural shock.

He found that only an inflammation followed putting the iodine into the pleural cavity. Serious nervous symptoms and death occurred only when the lungs were injured, and the use of iodine allowed its easy detection in the central nervous system — cerebral emboli of iodine. Similar results followed the injection of the tincture into the carotid arteries, and these in their turn were duplicated by the injection of air into the same vessels. When the pneumogastric nerves were cut, results were the same.

GEORGE RUKSTINAT.

A DEATH FROM SAPROVITAN. T. COHN, Deutsche med. Wchnschr. **53**:1048, 1927.

Living saprophytic bacteria injected intravenously have been used for several years under the trade name saprovitan principally for chronic diseases of the central nervous system. The preparation is made in Saxony, and its use has been confined mainly to European countries.

Seventeen days after the last of twelve injections given about twice a week for multiple sclerosis in a man, aged 34, death resulted from sepsis accompanied by jaundice. A bacillus of the coli-lactis group was obtained from the preparation used and also from the patient's blood. Cohn mentions five other cases of poisoning from saprovitan with three deaths.

E. R. LE COUNT.

BLOOD GROUPS OF CONVICTS. M. GUNDEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **11**:99, 1928.

From any statistics on the blood grouping of citizens and of convicts in Schleswig-Holstein, decided differences appear in the percentage distribution among the four blood groups which cannot be attributed entirely to racial influence. Among the criminal class group AB is seldom found, while group B is remarkably frequent. Group B is also frequent among the women convicts (especially prostitutes) and persons convicted of a second crime. No attempt was made to draw definite conclusions from so few figures obtained within so limited a territory.

ETHEL B. PERRY.

A New Agglutinin-Concentration Method for Determining the Group of Old Dried Blood. W. A. Müller, Deutsche Ztschr. f. d. ges. gerichtl. Med. **11**:120, 1928.

Dried blood (from 50 to 100 mg.) was extracted in from 0.2 to 0.3 per cent sodium chloride solution (from 20 to 30 cc.), which was found preferable to both distilled water and physiologic salt solution, for twenty-four hours in the icebox, and the filtered extract evaporated in vacuum at 20 C. to dryness, or a syrupy consistency was mixed with 0.5 per cent salt solution, equal parts or two parts salt solution to one of dried extract to make an isotonic concentrated solution of the agglutinins. Two drops of this extract were then tested with two drops of a 2 per cent lecithin-erythrocyte suspension, of A and B cells, respectively, and a macroscopic reading of the agglutination made in from ten to sixty minutes. In 70 per cent of eighty specimens of blood, dried for from one half to eighteen months, definite group determination by this method was obtained. The grouping of dried blood is consequently suggested as an acceptable medicolegal procedure.

Ethel B. Perry.

Imprint and Abrasion Revolver Wounds from Contact at the Time of Discharge. A. Werkgartner, Deutsche Ztschr. f. d. ges. gerichtl. Med. **11**: 154, 1928.

Many automatic self-reloading revolvers have a second barrel vertically in line with the channel through which the bullet passes. In some, this additional chamber is above, in others below that for the passage of the bullet. For that part connected with reloading, there is a spring operated by the discharge of the bullet by which in some such weapons a bolt is thrown back and forth in this second chamber; in others, the solid metal piece about both barrels slides back and forth as the weapon is reloaded.

The wounds made by such firearms when the muzzle is held against the skin as they are fired are of great significance, because with the reloading rebound which occurs practically simultaneously with the escape of the missile, an imprint-bruise is made of this second or reloading chamber, or else of the tip of the entire metal casement for both chambers. It is thus possible to obtain important clues to the make of the revolver and, what is of perhaps greater importance, the way the weapon was held when fired.

When reloading is effected by a bolt in a fixed chamber, the bruise close to one edge of the bullet hole is ring-shaped. When, on the other hand, the metal surrounding both barrels moves forward and backward, the oblong bruise takes the shape of the entire front end of the weapon. Of course, many such wounds are suicidal, and the revolver is found by the body. Sometimes the weapon is removed before investigation has begun. Werkgartner relates a number of interesting cases with excellent photographs of the wounds, illustrations of the weapons, and of the wounds of the hands which are prone to occur with these self-reloading revolvers.

E. R. Le Count.

Tumor and Trauma. Steida, Zentralbl. f. Chir. **54**:1070, 1927.

The following are again mentioned as important for a causal relationship between trauma and tumor development: absence of tumor anywhere and good health at the time of injury; no previous disease at the place injured; growth of the tumor at the place where violence is applied; proof of injury; some considerable degree of injury; appearance of the growth within a reasonable time after the injury and continuity of symptoms bridging over the interval between the two.

The long continued mild and repeated injuries responsible for carcinoma and the single violent injury from which sarcoma sometimes results, are emphasized, also that glioma of the brain may follow injuries of the head.

The warning is also expressed that dogmatic assumption of the truth of some of the assertions which have been made should not be carried too far.

E. R. Le Count.

Incidence of Fatal Postoperative Pulmonary Embolism. Oehler, Zentralbl. f. Chir. **54**:939, 1927.

Surgeons of northwestern Germany at a meeting in Hamburg in December, 1926, stated that the increase of deaths from postoperative pulmonary embolism was due to too much intravenous therapy. Some of them reported fewer such deaths when intravenous injections were discontinued. These points were brought out in the discussion which followed the report by Oehler of ten deaths. There was mention also of a total of about 93,000 operations with fatal embolism following in from 0.14 to 0.54 per cent; of its greater frequency after abdominal operations in women and of its great infrequency before the age of 30 years.

E. R. Le Count.

Technical

An Efficient and Rapid Method of Concentration for the Detection of Ova and Cysts of Intestinal Parasites. D. de Rivas, Am. J. Trop. Med. **8**:63, 1928.

The advantages and imperfections of various laboratory methods used in the past for the detection of ova and cysts of intestinal parasites are discussed. The author then describes his own method which is one of concentration by centrifugalization after the material has been treated with dilute acetic acid. The method is rapid and practical, requiring little time and equipment. Besides being useful in the detection of ova, cysts and other small bodies of clinical importance, it is also advantageous in testing for occult blood and in the determination of the bile content of the feces.

Pearl M. Zeek.

Determination of Cholesterol in Small Amounts in Blood. S. M. Ling, J. Biol. Chem. **76**:361, 1928.

The sample of blood is pipetted onto strips of filter paper, dried and submitted to continuous extraction in an especially designed micro-apparatus, and the cholesterol content of the extract is determined colorimetrically.

Arthur Locke.

Determination of Blood Sugar. S. R. Benedict, J. Biol. Chem. **76**:457, 1928.

A new and improved technic is presented for the determination of blood sugar which appears to indicate the true dextrose content of the blood more closely than the procedure of Folin and Wu. Analyses of forty samples of human blood have failed to indicate the presence of appreciable quantities of a fermentable sugar other than dextrose.

Arthur Locke.

Accuracy of Quinhydrone Electrode for Determining p_H of Blood Plasma or Serum. G. E. Cullen and I. P. Earle, J. Biol. Chem. **76**: 565, 1928.

Comparison of Colorimetric Method with Hydrogen and Quinhydrone Electrodes. Ibid., p. 583.

A method is described for the potentiometric determination of the hydrogen ion concentration of the blood, the quinhydrone being used in place of the hydrogen electrode. The values obtained are accurately reproducible but appear to be consistently 0.06 p_H more acid than the values obtained with the hydrogen electrode.

Estimation of the p_H of normal serum by the colorimetric method gives values which are constantly 0.08 p_H higher than those obtained by the use of the hydrogen electrode and 0.14 p_H higher than those obtained with the quinhydrone method.

ARTHUR LOCKE.

THE TECHNIC OF OPERATIONS ON MICE. WERNER KOOSE, Centralbl. f. Bakteriol. **106**:140, 1928.

This paper, with several illustrations, outlines the general technic in certain operations on mice, with suggestions as to methods of value in this type of experimental surgical procedure.

PAUL R. CANNON.

Society Transactions

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HARRISON MARTLAND, *President, in the Chair*

A CASE OF PERIARTERITIS NODOSA IN A BOY TEN YEARS OF AGE. SARA WELT.

Periarteritis nodosa appears to be a rare and not well known disease. There are only thirteen cases tabulated in the American literature. Three of these were reported before the New York Pathological Society, two from the Presbyterian Hospital by A. R. Lamb, one in 1913 and the other in 1917, in a girl 10 years of age. In 1919, Manges and Baehr added a third case from Mt. Sinai Hospital.

The case reported is that of a previously healthy boy (the boy was presented at the clinical conference of Mt. Sinai in February, 1924, under the caption of "A Case for Diagnosis"; a tentative diagnosis was made of periarteritis nodosa), aged 10 years, who was admitted to the pediatric service of Mt. Sinai Hospital on Oct. 2, 1923, with a history of having had pain in both upper and lower extremities for the last ten weeks; during the last four weeks before admission he was in bed on account of increasing pain and fever; on admission the boy appeared to be rather poorly developed. The skin showed no eruption; the pharynx mucosa was injected; the tonsils were enlarged; the organs of the chest appeared normal; the pulse rate was accelerated. Results of examination of the abdomen were negative; the spleen was just palpable; any attempt of motion of both upper and lower extremities was painful; the joints were not involved; the urine was normal. The blood showed a leukocyte count of 14,000, with 80 per cent polymorphonuclears. During the first four months of the patient's long stay in the hospital, the most prominent symptoms were pain in the muscles of both the upper and the lower extremities leading to atrophy of the muscles with some contractures; the joints were not involved. A small piece of muscle from the right arm, excised for biopsy, showed dilatation of vessels with perivascular infiltration of polymorphonuclear cells; the muscle fibers were normal. The patient had a continuous fever up to 103.2 F. with an accelerated pulse rate. There was some leukocytosis with occasional polynucleosis. The urine was generally normal and on only a few occasions showed traces of albumin and occasional white blood cells; the blood chemistry showed normal figures; the blood culture was negative; the blood and cerebrospinal fluid Wassermann tests were both negative. The blood pressure was increased during the last two months of his sickness, the systolic pressure varying between 145 and 170, and the diastolic between 112 and 135. Examination of the stool did not show the presence of ova or parasites. X-ray examination of the thorax, pelvis and both upper and lower extremities failed to show any definite evidence of abnormality.

Beginning in the fifth month after admission, the patient was seized with repeated severe attacks of general convulsions with loss of consciousness and incontinence of feces and urine. Lumbar puncture made repeatedly elicited clear fluid under normal pressure; the cell count was not increased. He gradually recovered from these seizures with vastly deteriorated sensorium and loss of memory. He would bite his fingers, actually chewing off the tips of the index and the two middle fingers of the right hand; six weeks before death he developed a left-sided orchitis, with swelling of the testicle and great sensitiveness on palpation. Death occurred from a terminal pneumonia on March 27, 1924.

From the symptoms presented, the case reported belongs to the neuromuscular type of periarteritis nodosa; noteworthy besides the long duration of fever and accelerated pulse rate is the paucity of cardiac and kidney symptoms when com-

pared with the extensive lesions present in these organs after death. Also noteworthy is the cerebral involvement with orchitis both of which were observed in a few cases only.

Necropsy was performed by Dr. George Baehr. The body was that of an emaciated boy, with marked atrophy of the muscles of the upper and lower extremities, and no subcutaneous nodules. The heart weighed 250 Gm. It was slightly larger than normal. The cavities and valves were normal, as was the amount of fluid in the pericardium. The appearance of the heart was striking and unusual. Along both coronary vessels, especially the descending rami on both anterior and posterior aspects of the heart, were numerous protuberances varying in size from that of a pinhead to that of a pea, the largest being the size of small grapes. They were reddish or bluish-red and apparently represented sacculated aneurysms. They were arranged like a set of irregular sized beads along the branches of the coronary arteries; the veins were clearly to be seen beneath the pericardium, running parallel to these chains of aneurysms and were entirely uninvolved in the process.

The kidneys together weighed 180 Gm. and were of about normal size; the capsules stripped easily, revealing a surface of remarkable appearance. The pinkish organ was irregularly mottled with small brownish-yellow, slightly depressed areas of fairly sharp outline. On section, they appeared to extend more or less deeply into the renal cortex; there were also some finer yellowish patches deeper in the cortex. They all appeared to be areas of necrosis; the only vessels showing evidence of disease were the interlobular branches which here and there appeared to be irregularly thickened.

Evidence of vascular changes was also found in the liver, suprarenals, stomach and small intestine.

Microscopic examination showed widespread lesions characteristic of periarteritis nodosa in most of the organs and tissues.

A complete report of this case will follow.

DISCUSSION

GEORGE BAEHR: After Dr. Welt's complete presentation of this subject, there is little left to add, and my only right to speak at all on the subject is because I happened to have reported one of the thirteen cases in the American literature. But the fact that there were only thirteen cases in the American literature up to last year is not an indication of the incidence of the disease. It is probably not uncommon, but clinically is not generally recognized, and is therefore regarded as a rare disease. As a matter of fact, our own case would never have been reported because shortly before that Dr. Lamb had made his contribution to the subject and had given an excellent review of the cases reported to that time. There would have been no reason to report our case had it not been that we had the unique opportunity of studying the arterial lesions from the onset of the disease until its termination.

The onset of the disease was characterized by abdominal symptoms so severe as to indicate the necessity for operation. An affected branch of the superior mesenteric artery was removed for microscopic study. For this reason we were able to secure the earliest lesions. Later, when the superficial lesions appeared we were able to study them, so that we had the opportunity to observe the progressive change in the microscopic lesions including the end-stages of the process seen at autopsy.

The earliest changes were characterized by an acute inflammatory lesion of the vessel wall, an almost pure polynuclear cell infiltration of the media, and to some extent of the adventitia. When extensive destruction of the media and elastic tissue of the wall had occurred, nodular bulgings of the wall sometimes occurred. By the time the patient died, many months after the first observation, all traces of the acute inflammatory process had disappeared, and in spite of the extensive stretch of the vessels involved, the lesions were purely those described by

the early investigators, Kussmaul, Meyer, and others—a degenerative type of lesion. There was no trace of the previous inflammatory process. In that way we were able to correlate the two types of vessel lesion which had previously been described, and to demonstrate that the so-called degenerative lesion was merely the end-result of the acute inflammatory process which had initiated the disease.

PAUL KLEMPERER: I would like to emphasize that it seems to me not a mere accident that we read now so frequently papers on periarteritis nodosa. The scope of the conception of periarteritis nodosa has widened considerably, and we do not consider only those cases as periarteritis nodosa which show the characteristic gross lesions described by Kussmaul, but also such cases in which only microscopic lesions have been found. I think it has been claimed by several authors that the lesions found in acute infectious diseases are similar or even identical with the lesions of periarteritis nodosa. Whether one should go that far, I do not know. I would not go that far, because the lesions as described by Wiesel and Wiesner in acute infectious diseases do not conform with the lesions of pariarteritis nodosa. There are, however, other conditions in which lesions are found which do resemble periarteritis nodosa under the microscope. Here I refer particularly to the lesions which one finds occasionally in renal diseases, in subacute nephritis as well as in the type which is called malignant sclerosis. In the first type, in subacute nephritis, one encounters not rarely, in 10 or 20 per cent of the cases, changes in the smallest arteries which are characterized by necrosis of the media. These changes are well known. It is not so well known, however, that there are also lesions of necrosis of the media in the larger arteries, and in those of the size of the intralobular arteries. I have seen one case of this type some years ago in a typical subacute nephritis of a stormy course (Löhlein), a boy, aged 4 years, in which lesions in the hilum of the kidney were found which could only be called periarteritis nodosa. There was the characteristic fibrinoid degeneration or hyalinization of the media with the characteristic inflammatory reactions in the adventitia. In the other organs there was no change. Lately I have seen a case similar to it in which the lesions were in the arcuate and in the intralobular arteries. Here the characteristic changes in the media were definitely to be seen. It is the necrosis which characterizes the arterial lesion to be found in malignant sclerosis, and I think in studying the cases of periarteritis nodosa which we have accumulated at Mt. Sinai Hospital one finds that the lesion in the media is also necrosis, or at least necrobiosis. It would be interesting to compare furthermore the lesions which have been described by VonGlahn and Pappenheimer in rheumatic diseases with the lesions of periarteritis nodosa. There are certain morphologic similarities, and if one accepts the hypothesis of Gruber on the pathogenesis of the lesions of periarteritis nodosa, one can possibly find some analogies between periarteritis nodosa as an hyperergic condition of the vessel wall, and rheumatic arterial disease, in which the lesion in the vessel occurs particularly in the recurrent type of rheumatic disease. I think the problem of periarteritis nodosa is not confined to the macroscopic and microscopic lesion alone; it is of a much wider scope.

BELA SCHICK: I can talk only about the clinical part of the picture. One cannot make the clinical diagnosis with any certainty. One can only guess at it from the clinical symptoms, because as Dr. Welt mentioned, there are these bizarre symptoms dependent on the part of the system affected. There are different types of circulatory changes occurring during lifetime. There is no doubt that we have to think of the diagnosis of periarteritis nodosa in cases in which the clinical picture is composed of nervous symptoms, atrophic muscles, which are influenced by poor nutrition of the arteries, and besides the nervous symptoms, nephritic symptoms and high blood pressure. I will make the same mistake the next time I see a case, but we should think of the possibility of this condition when we find a bizarre or peculiar picture of symptoms which cannot be explained in other ways. In this one case we thought of polymyositis, also some parasitic disease, like trichinosis, because the pains were so marked in the muscles, and there were

10 per cent of eosinophils in the blood. The diagnosis can be arrived at only by taking out a piece of an artery during life. Usually it will not be made until after death, or, rarely at operation.

SARA WELT: I believe with Dr. Baehr that the disease is probably not rare. I can easily imagine that patients with minor lesions are treated for rheumatism, neuritis or other conditions.

I agree with Dr. Schick that clinicians should think of the possibility of periarteritis nodosa in cases with bizarre symptoms, which cannot be brought under one head. The diagnosis *intra vitam* can be made only by excision of nodules for microscopic study.

THE SIGNIFICANCE OF POSTMORTEM BACTERIOLOGIC EXAMINATIONS WITH SPECIAL REFERENCE TO STREPTOCOCCI AND ENTEROCOCCI. EMANUEL Z. EPSTEIN and M. A. KUGEL.

This study was undertaken to ascertain the value of bacteriologic investigations at necropsy. The blood was examined in 66 instances, the bone marrow in 62, the heart muscle in 42 and the heart valve in 40. The valves were without any gross inflammatory changes in 35 cases.

In all the cultures of the blood and bone marrow at least one organism was present. In the heart valve there were four and in the heart muscle five sterile cultures. Streptococci were found in 80 per cent of the cultures of the blood, in 67 per cent of the bone marrow, in 47 per cent of the heart muscle and in 40 per cent of the normal valve. Fourteen of twenty-six strains of *Streptococcus alpha* or *Strep. viridans* tested were identified as enterococci. Of six strains of *Streptococcus beta* or *Strep. hemolyticus* tested, none were enterococci, and of thirty-five strains of *Streptococcus gamma* or *Strep. anhemolyticus,* thirty-two were identified as enterococci.

Bacillus coli was recovered in 42 per cent of the blood cultures, in 51 per cent of the bone marrow, in 50 per cent of the heart muscle and in 52 per cent in the heart valve. *Bacillus pyocyaneus* was isolated in 21 per cent of the blood cultures, in 30 per cent of the bone marrow and in 10 per cent of the heart muscle. *Staphylococcus aureus* was obtained in 13 per cent of the blood cultures, in 11 per cent of the bone marrow, in 14 per cent of the heart muscle and in 15 per cent of the heart valve cultures.

There was a general agreement between the observations on the bone marrow and blood. One interesting exception was a case of a subacute bacterial endocarditis in the bacteria-free stage with negative antemortem cultures, in which *Streptococcus alpha* or *viridans* was recovered only in the bone marrow.

Streptococcus beta or *Strep. hemolyticus* was found infrequently in the blood cultures taken post mortem (five times in sixty-six cases). There were three cases of chronic pulmonary tuberculosis and two of general peritonitis.

The pneumococcus was found in only one instance of the sixty-six blood cultures at necropsy, a case of pneumococcus pneumonia. Although only two cases of lobar pneumonia were available, it is of particular interest that the pneumococcus was not once recovered at necropsy in the other sixty-four cases.

The question as to whether the bacteriologic observations represent an agonal or a postmortem invasion can be definitely settled only by further studies in which blood cultures are taken shortly before death and again at necropsy. In a few instances in our cases there were blood cultures taken during the last thirty-six hours of life which were negative and which at necropsy revealed streptococci.

The recovery of streptococci in 40 per cent of normal valves, of which 25 per cent were *Streptococcus alpha* or *viridans,* is exceedingly interesting in view of the great importance that has been attached to the presence of bacteria in the macerated valve in rheumatic endocarditis. From the presence of *Streptococcus viridans* in the valve cultures of all of the eight cases of verrucous endocarditis, Reye lays a causative importance to this organism in the etiology of the disease.

The identification of many strains of streptococci as enterococci would possibly indicate that the source of the invasion was mainly from the intestinal tract. And the occurrence of enterococci in the blood, bone marrow, heart muscle and valve in the most diverse cases makes it seem advisable to study both during life and at necropsy the streptococci found in rheumatic fever and other diseases in order to determine the frequency of the occurrence of enterococci and to gain more knowledge of their rôle in the production of disease.

Our results would indicate that only when unusual organisms are recovered after death are they of definite diagnostic value. And it seems that no significance can be attached to the presence at autopsy of *Streptococcus alpha* or *viridans*, *Streptococcus gamma* or *anhemolyticus*, *Staphylococcus aureus*, *Bacillus coli* and *Bacillus pyocyaneus* unless the same organism has been found during life.

DISCUSSION

GREGORY SHWARTZMAN: The point of most interest in this work lies in the fact that the streptococci and other organisms can be found in postmortem cultures in cases in which bacteria cannot be found antemortem. Especially interesting is the presence of the streptococci.

In connection with the source of the invasion I want to point out the significance of the observation for enterococci. *Streptococcus faecalis*, which is supposed to be a normal inhabitant of the intestinal canal, was classified as an enterococcus. That is, the enterococcus is probably a subgroup of *Streptococcus faecalis*. The characteristics of the enterococcus are specific; therefore while it is difficult to classify *Streptococcus faecalis* as such, it is easy to determine the enterococcus. The special characteristic of the enterococcus is the fermentation of esculin. It is interesting that the percentage of streptococci which were able to ferment esculin was insignificant in antemortem bacteriology. Recently Dr. Levinson had occasion to study in our laboratory 125 strains of *Streptococcus hemolyticus*, out of which four were able to ferment esculin. When we looked at the pedigree of these four strains, we found that three were of postmortem origin, and that one was isolated during life but came from a scrotal abscess; so it is definite that the classification of streptococcus as an enterococcus might be of importance in determining the origin of the organism during the course of infection.

The other point of interest is the inability to find hemolytic streptococci as postmortem invaders. *Streptococcus viridans* or *gamma*, or *B. coli* were found. *Streptococcus hemolyticus* or pneumococcus was found infrequently. That is curious. The intestinal and upper respiratory tracts contain *Streptococcus hemolyticus* under normal conditions, and there is no reason why it should not invade as well as *Streptococcus viridans*. To explain that point I should like to bring out an observation of Maximow in tissue cultures. He cultured rabbit leukocytes with bovine tubercle bacilli, and another set with human tubercle bacilli. The human tubercle bacilli multiplied speedily, while the bovine type multiplied more slowly. We know well that the human strain is less pathogenic for the rabbit tissue than the bovine is, and evidently the pathogenicity of the organism has some relation to the proportion of invasion.

In discussing the source, or the time of invasion, I would like to say that I personally believe that invasion is not postmortem. It occurs probably a short time before death. This belief I base on the fact that if the organisms do invade from the intestinal canal, it is difficult to understand how shortly after death they would be found in the bone marrow, heart muscle, and in distant parts, because for invasion to occur after death, we would expect that there would be a continuous film of growth extending from these parts into the different areas. It would be impossible to conceive that such a bacterial growth could occur in a short time. Evidently, there must be some circulatory force which sends out the bacteria from the points where they are contained shortly before death. I believe the invasion of bacteria occurs not only shortly before death, but during life, and this

point was brought out by Dr. Epstein in connection with the work on muscles of living animals he quoted. There are some other observations which might point to true transient bacteremias not associated with thrombi throwing out bacteria in the blood. The observation referred to was made in Denmark where blood cultures were taken on normal horses used for the preparation of antitoxin. Large quantities of blood were taken, and shortly after a heavy meal the horses had a septicemia for an hour or so, and then when the meal was digested, the blood became normal again. During the last few years I have had occasion to observe a number of positive blood cultures of short duration. Clinically, there was no suggestion of bacteremia or septicemia. The symptoms were insignificant, except a pyrexia for a day or two. Blood cultures frequently showed streptococci in such cases. The following blood cultures were negative. This occurs in debilitating diseases, like carcinoma, ulcerative colitis and others. It is interesting that the type of invader always found is *Streptococcus viridans*, but never *Streptococcus hemolyticus*. The latter is always associated with a real septicemia.

The absence of *B. welchii* in postmortem bacteriology in man is evidently the same as in animals. In order to find *B. welchii* one must inject *B. welchii* into a rabbit and then kill it, and after twenty-four hours' incubation of the dead rabbit, one may find the organisms, but if the organisms are not injected, they will never be found. There is infrequent invasion of *B. welchii* post mortem.

EMANUEL LIBMAN: As a result of studies on nonhemolytic streptococci, I had taken it for granted that they would frequently be found in postmortem investigations. The figure obtained by Dr. Epstein and Dr. Kugel is, however, higher than I had anticipated that it would be practicable to obtain. In 1923, in a paper on endocarditis, I discussed the conditions apart from subacute bacterial endocarditis in which I had found nonhemolytic streptococci in the blood. I pointed out that they may be found as secondary or terminal invaders in the greatest variety of diseases, even in patients who are not in poor condition.

Nonhemolytic streptococci may be found in the blood in cases of valvular disease, without there being any active endocarditis present, and they may be encountered as secondary invaders in a case of endocarditis due to another organism. These organisms are the most important secondary invaders with which we have to deal, and not the colon bacillus, as we used to believe. They are really to be regarded as ubiquitous. Sir Thomas Lewis, in one of his papers published in association with Grant, also stated his opinion that such cocci were continuously invading the body.

On the basis of these data, I believe with Dr. Shwartzman that the invasion most likely occurs before death. Dr. Epstein had intended to note that streptococci have also been found in the upper air passages. One therefore must allow that the invasions may originate there, as well as in the intestinal tract.

One of the most important studies on the invasion of bacteria during meals was made by Adami. This was known to occur in animals, and Adami suspected that it occurred in human beings, because he found in the liver what he considered to be coccoid forms of the colon bacillus. As far as *B. welchii* is concerned, I believe that in order to come to a final conclusion it will be necessary to make the Welch test on rabbits with postmortem blood in a fair series of cases. I have evidence which points to the fact that antemortem invasions by *B. welchii* occur in the course of chronic disease or following abdominal operations, and that these are not particularly rare. In one such case the lungs showed hemorrhagic areas full of the bacillus, the heart blood revealing none in spreads or culture, but responding positively to the Welch test.

The enterococcus interests me particularly because the question has arisen whether or not it is the same as the streptococcus described by Hirsh and myself under the direction of Escherich. I had never made use of the designation Hirsh-Libman streptococcus, but the recent literature has revived it. In the article on the

enterococcus, contributed by Professor Nissle of Freiburg to the new edition of the Kolle-Wassermann (Uhlenhuth) Handbuch, it is stated that the enterococcus is identical with *Micrococcus ovalis* of Escherich, the Hirsh-Libman streptococcus and *Streptococcus faecalis,* and not definitely with *Streptococcus lactis.* As I have not made studies of my own on these relationships, I can give no opinion concerning the correctness of these views.

It is evident from these studies of Drs. Epstein and Kugel and from other investigations that organisms corresponding to alpha, beta and gamma streptococci may all have the characteristics of the enterococcus, this designation being applied to organisms that are usually lancet-shaped, and have the other characteristics mentioned tonight. Their investigations emphasize the necessity of studying all streptococci recovered during life, as well as after death, in order to ascertain the real incidence of enterococci and what special rôle they actually play.

Louis Gross: This study of Drs. Epstein and Kugel is a rather severe indictment against some of the recent work on the etiology of rheumatic disease. Some of these papers have described studies in which nonhemolytic streptococci were obtained from feces, tonsils and other sources. Unfortunately most if not all of these authors have not stated in their papers whether they used the bile, heat and esculin tests to determine whether their organisms were enterococci or not. In the absence of these tests these organisms have been accepted on slender grounds as the etiologic agent of rheumatic disease. The observations of Kugel and Epstein of these organisms in such a large number of perfectly normal valves in cases in which there had been no history of rheumatic disease throw considerable doubt on this type of work.

Dr. Epstein: In answer to the question of whether these cultures represent an agonal or postmortem invasion, I would like to quote two cases. One was a case of a pneumococcus type IV meningitis in which a blood culture three hours before death showed *Streptococcus alpha* or *viridans.* Another was a case in which an intracardiac culture was taken five minutes after death and in which an enterococcus was recovered. It is inconceivable that an enterococcus five minutes after death could have been a postmortem invader. These two cases seem to point to an agonal invasion.

Lesions of the Pulmonary Artery and Pulmonary Valve in Rheumatic Disease. M. A. Kugel and Emanuel Z. Epstein.

The present study was undertaken to determine the frequency and type of inflammatory changes in the pulmonary artery and valve and in the adjacent fibrous ring in cases of rheumatic heart disease.

The fifty-nine cases of rheumatic cardiac disease studied have been subdivided into two groups: first, cases (twenty-four in number) in which there was clinical evidence of active rheumatic infection and the presence at autopsy of acute verrucous endocarditis, fibrinous pericarditis or Aschoff bodies in the myocardium, and second, cases (thirty-five in number) of chronic rheumatic cardiovalvular disease in which there were no signs clinically or pathologically of active rheumatic infection.

All the patients in the rheumatic group, except one, gave a negative syphilitic history and a negative Wassermann reaction. Cases of rheumatic cardiac disease complicated by acute or subacute bacterial endocarditis were not included.

As controls we studied the pulmonary artery and valve in two groups of cases, first thirty-one cases of pericarditis not rheumatic in origin, and second seventy-five routine autopsy cases with neither pericarditis or evidence of rheumatic infection. Unfortunately the latter did not include cases of acute infectious diseases such as typhus fever, typhoid fever, scarlet fever and diphtheria. Such a group would be valuable for control examination, since these infections might conceivably produce lesions in the artery more or less resembling those in the rheumatic cases.

The sections of the pulmonary artery were taken to include the artery, valve and myocardium at the root of the artery, and at various sites up to the insertion of the vessel into the lung.

In the twenty-four cases with evidences of active rheumatic infection, Aschoff bodies were present in the myocardium in nineteen, an incidence of 79.2 per cent.

Lesions were found most frequently at the root of the pulmonary artery, where the artery and valve are inserted into the fibrous ring. At this point the pulmonary artery sends out strands of connective tissue between the muscle fibers, and we have designated this area the musculo-arterial junction. This area was involved in seventeen of the twenty-four instances. Two types of reaction were noted, one consisting of Aschoff nodules and the other of diffuse cellular infiltrations composed of lymphocytes, polymorphonuclear leukocytes, large mononuclear wandering cells, and multinuclear cells of the kind seen in the Aschoff bodies. This diffuse reaction was the one more regularly observed. It is often conspicuous, extending from the adjacent myocardium into the fibrous ring and into the base of the artery and valve.

In five of these twenty-four cases of active rheumatic cardiac disease, histologic examination of the artery revealed active inflammatory changes consisting on the one hand of diffuse cellular infiltration in the intima and subintimal layers of the media and on the other of focal perivascular collection of cells comparable to the Aschoff nodules in the myocardium. In two instances the intimal changes were sufficiently widespread to produce small macroscopic lesions on the inner surface of the vessel. In two cases the disruption of the elastic layers of the media was almost as conspicuous as in syphilis. In fourteen of the active cases, the pulmonary valve was the seat of an interstitial valvulitis which involved a part or the whole of the valve, and in three of these the diffuse reaction was accompanied by Aschoff bodies in the substance of the valve. Verrucae were present in six cases, but these were inconspicuous in all but two.

In a comparative study of the aorta, its root and valves, the fibrous ring of this vessel which corresponds to the musculo-arterial junction of the pulmonary artery was involved in twenty-two of the twenty-four cases of active rheumatic heart disease or 90.7 per cent. The aortic valve was involved in twenty-one of the twenty-four cases, and in three instances there were gross lesions in the intima similar to those described by von Glahn and Pappenheimer. Inflammatory changes of the diffuse type were found in the wall of the artery in each of the cases with intimal lesions.

In the thirty-five cases of chronic rheumatic heart disease without evidences of active infection we occasionally found scarring and small lymphocytic foci in the musculo-arterial junction and disruption and scarring in the media of the pulmonary artery. We did not observe any of the acute lesions seen in the active cases.

In the control cases consisting of thirty-one of pericarditis of other than rheumatic origin and seventy-five cases in which neither pericarditis nor rheumatic disease was present, except for scarring of the media, there were not found lesions which could be confused with those seen in the pulmonary artery, valve and musculo-arterial junction, in cases of active rheumatic cardiac disease.

(The complete paper will appear in the ARCHIVES OF PATHOLOGY.)

DISCUSSION

EMANUEL LIBMAN: Balfour said many years ago that the area of the pulmonary artery was the area of romance. I have always considered it the area of fascination in the study of cardiac disease, and that is why I personally welcome this contribution that makes a valuable addition to the studies of von Glahn and Pappenheimer, which we may already consider classic. The pulmonary valve and artery are liable to a great variety of diseases, ranging from gross congenital defects to the so-called idiopathic dilatation of the pulmonary artery, and the dilatation that one at times notes in cases of partial or complete obstruction of the portal vein.

There are new fields of study and speculation opened up by the studies just presented. One is the relationship of the changes to chronic disease of the pulmonary artery. Another refers to the auscultatory and clinical phenomena to which they may give rise. Kugel and Epstein have given a hint concerning an auscultatory sign.

It is, of course, probable that atherosclerosis and deposition of calcium may occur in the lesions that they have described, and also thrombosis. It may well be that those cases of mitral stenosis that show the more marked chronic changes in the branches of the pulmonary artery may be those in which there were first present the rheumatic vascular lesions.

Comparatively little systematic study of the pulmonary artery has been made. There do exist elaborate investigations of the congenital lesions. Although we hear much of syphilis as the cause of Ayerza's disease, there are only few careful pieces of work on the subject. In the recent contribution made by S. Peck, it is stated that there are on record only about fifteen cases of syphilis of the pulmonary artery that can be accepted.

I want to speak of another subject which has been of interest to me and which gives a reason for a restudy of at least the systolic murmurs heard on the left side of the chest. It was pointed out many years ago by Meyer, Skoda, Bamberger, Gerhardt, Naunyn and others that the murmur of mitral insufficiency is not infrequently heard better at the base of the heart, especially over the pulmonary artery, than at the apex. Further, it was found that the murmur might be heard earlier at the base and then later at the apex.

My experience with calcification in the heart may throw light on this subject. I have noted that when calcification of the mitral ring or calcification in the aortic flap of the mitral valve is present, a systolic murmur, if present, is apt to be heard louder to the left of the sternum than at the apex, or be heard only in the former location. This observation and the studies presented tonight give us two possible explanations of the peculiar localization of the systolic murmurs of rheumatic mitral insufficiency.

The first possibility, perhaps the one that is less frequently important, is that a patient may be suffering from a rheumatic involvement of the pulmonary artery which heals before or while a mitral insufficiency is developing. The second is that the murmur in the pulmonic area is due to mitral valvulitis and that it may diminish or disappear if the valvulitis lessens or disappears while an insufficiency develops due to disease of the free margins of the cusps of the valve. It is suggestive that Kugel and Epstein have found rheumatic changes in valves to be most marked at the ring.

WILLIAM VON GLAHN: I am glad that Kugel and Epstein have reported this study, because it seems to emphasize clearly several important points. In the first place, as is well recognized now, the initial damage in the valve is an acute interstitial valvulitis; second, rheumatic disease is a disease of the cardiovascular system; third, in the blood vessels themselves there may be produced a lesion which can be recognized in the gross and which can be separated readily from syphilis, on the one hand, and ordinary arteriosclerosis, on the other, and finally, the Aschoff nodule is not the only characteristic histologic lesion of rheumatic disease. There is a more acute, more diffuse, and a much more widespread reaction than the Aschoff nodule, and this reaction is just as characteristic as the Aschoff nodule.

M. A. KUGEL: Scarring of the media of the pulmonary artery is not uncommon in conditions other than syphilis or rheumatic fever.

Aside from the result of a localized destructive inflammatory process in the wall of the vessels, it is possible that scarring may be due to nutritive disturbances, as suggested by Scott. He has noticed in syphilitic aortitis that part of the early lesion was an endarteritis of the vasa vasorum, with obliteration of lumina in some instances.

The vasa vasorum, especially those in the adventitia of the pulmonary artery, in our cases of rheumatic carditis, in some instances showed evidences of arteritis,

marked thickening of the muscular wall, proliferation of the intima and partial or complete obliteration of the lumina.

In a case of Hodgkin's disease in a girl, aged 18, the base of the pulmonary artery was compressed by an enlarged lymph node. The pulmonary artery in this case showed marked scarring which was probably due to nutritive disturbances to this area.

DEVELOPMENT CYCLE OF THE TUBERCLE BACILLUS AS REVEALED BY STUDIES ON A SINGLE CELL. MORTON C. KAHN.

Particular impetus has recently been given to the subject of the biology of the tubercle bacillus by members of the French School who have stated that filtrates of cultures of tubercle bacillus or filtrates of organic extracts from tuberculous animals or human beings after thoroughly controlled sterility tests will produce tuberculosis in varying degrees when injected into laboratory animals. Some of these French authors are Fontes (Centralbl. f. Bakteriol. **51**:244, 1912), Hauduroy and Vandremer (Compt. rend. Soc. de biol. **89**:1276, 1923), Arloing (Bull. Acad. de méd. Paris **36**:301, 1926), Calmette and Valtis (Presse méd. **90**:1409, 1926) and DePotter (Compt. rend. Soc. de biol. **96**:138, 1927). A number of observers in other countries have attested to the often encountered variations from the usual acid-fast rod, and in view of the existing evidence it cannot be taken for granted that the sole means of reproduction of the tubercle bacillus is by simple fission. Among these observers are Mallassey and Vignal (Arch. de physiol. norm. et path. **2**:369, 1883), Semmer (Deutsche Ztschr. f. Thiermed. **21**: 212, 1892), Arrigo (Centralbl. f. Bakteriol. **28**:481, 1900), Much (Beitr. z. klin. d. tuberk. **8**:357, 1907), Spengler (Ztschr. f. Hyg. u. Infectionskrankh. **49**:541, 1905) and Sweany (Am. Rev. Tuberc. **17**:53, 1928).

The observations of most workers in this field were made by studying stained preparations taken periodically from clinical material, from cultures of the bacillus of tuberculosis or from hanging drops made with substances obtained from similar sources. While interesting and highly suggestive, observations made on preparations stained or otherwise in which a large number of micro-organisms are encountered must be confusing when studies on the life cycle are attempted, especially if the types are not undergoing an identical phase of their development at the same time. Such conditions must seldom be the case. In examining preparations containing many bacteria in the living stage especially, it is difficult, if not, impossible, to keep the eye constantly affixed to a single organism, and if one glances away from the field, if only for a second, one cannot be sure of returning to the identical bacillus under original observation.

To obviate this difficulty, observations have been made on more than 200 preparations of single tubercle bacilli and on as many more preparations of small groups of three and four. These single organisms were grown, each in a separate microdroplet, under conditions which generally insured active growth, in such a manner as to maintain them in an actively viable condition for several days and often weeks. For, in contradistinction to the usually encountered pathogen such as *B. typhosus*, which undergoes a generation several times in twenty-four hours, it has been our experience that the strain of tubercle bacillus under observation develops from mature rod to mature rod at a much slower rate, about from every six to eight days, and some organisms have taken as long as twenty-six days to attain maturity.

The strain of tubercle bacillus used in this investigation was the H 37, a pathogenic human type obtained from the National Tuberculosis Association.

Stock cultures of this organism were kept going by transplanting onto Long's synthetic liquid medium. Luxuriant growth always ensued in a minimum of time, and this substance was found equal if not superior to the egg mediums or glycerin broth for supporting growth.

The medium used to grow the single cells and small groups was Long's medium, but in addition 0.2 per cent of agar and 1.5 per cent of gelatin. These last named

ingredients were added to prevent the microdroplets from spreading and mixing with the moisture of condensation deposited on the underside of the coverslips, and it was this modified and viscid medium which made it possible to retain the droplets in perfect condition for such relatively long periods of time. Liquid medium has been used heretofore for the isolation of single cells, but it was found to be unsuitable for the present study.

The stock culture was incubated in the agar gelatin mixture for four days prior to isolating single cells, and in this way a number of free rods were obtained without having to shake the mass obtained from the mother culture.

The droplets were placed on the underside of a scrupulously cleaned and sterile no. 1 cover slip mounted on the Chambers cell, which is a glass box open at the top and one side. The isolation of the single bacteria and small groups was made possible through the use of Chambers micromanipulator, an apparatus devised for the purpose of manipulating micropipets in three planes of the field of a compound microscope. The pipets have openings of about 1.5 micron and thus are small enough so that tiny drops may be expelled often containing a single bacterium.

After four droplets containing single bacilli or groups of not more than four were isolated, the cover slip was removed from the glass cell and rapidly transferred to a deep hollow ground slide. The cover slip was then rapidly sealed in place with a hot petrolatum paraffin mixture standing close at hand. The droplets were successfully prevented from drying by placing a drop of sterile 3 per cent agar in the bottom of the depression slide before the cover slip was sealed in place. Next, the droplets were examined under the 2 mm. oil immersion with 15 X eye piece and accurate drawings made of the single cell or the small groups present in each individual droplet. The slides were incubated at 37.5 C. in petri dishes having moist filter paper on the bottom. These drops were small and shallow and thus permitted complete visibility of all parts; therefore, it was impossible to have an organism or granule in the drop and not be cognizant of its presence. A number of sterile droplets were observed, and in no case did contamination ensue. Observations on several slides thus prepared were made in the micro-stage incubator, and it soon became evident that a change did not take place in the individual rod during a few hours' incubation, aside from the sometime formation of central, polar or bipolar granules; therefore, subsequent observations were made at twenty-four hour intervals.

It is stated by authors of most textbooks on bacteriology that the tubercle bacillus multiplies by simple fission. Branching forms have been described by numerous investigators, but Petroff (Tuberculosis, Bacteriology, Pathology and Laboratory Diagnosis, Philadelphia, Lea & Febiger, 1927) considers these to be juxtaposition of two individual rods and claims that he has never seen true branching types of the organism. This is contrary to our experience, for on several occasions a second rod has been observed to sprout from an intrabacillary grandule and finally break from the parent form, the daughter rod being considerably shorter and finer. Such cases, however, are exceptional as are those in which a short rod has been observed to form between the dividing halves of a free granule or fragment, the dividing halves ultimately stretching apart and forming two small rods with polar bodies.

Under these experimental conditions the most frequently encountered development cycle of the tubercle of this particular strain has been as follows: After a few hours' incubation in the microdroplet, a single, unstained homogeneous rod will begin to form certain zones, which appear darker than the surrounding bacillary protoplasm. These dark areas are taken to be the deep staining portions which appear black or deep red when the tubercle bacilli are stained with Krylow's carbol fuchsin carbol methyl violet stain. Such zones are formed centrally, subterminally or at both poles. At times, as many as ten and on one occasion sixteen have been observed in a single rod, but for the most part two or three are present.

From about twenty-four to seventy-two hours after planting, and often longer, lines of cleavage become evident, but these are independent of the position of the

dark bodies already formed, for often a rod having bipolar bodies will form four zones of cleavage, two of them, therefore, being without the deeper appearing areas. As will be seen later, this particular type of cleavage is not simple fission in which a rod subdivides along a more or less central line, each resulting half developing to redivide once more, but a segmenting process giving rise most often to three or four separate units which remain in the form of oval bodies. The number of units thus formed is seemingly governed by the size of the rod. In this stage the coccoid bodies are acid-fast in agreement with Spengler's (Ztschr. f. Hyg. u. Infectionskrankh. **49**:541, 1905) description of his splitter, but at times nonacid-fast globoid forms are encountered, which are surrounded by a delicate acid-fast zone. These are possibly the portions of the rod which contained the dark appearing areas. These resulting coccoid types do not henceforth develop directly into adult bacilli, but many of them subdivide by simple cleavage along a more or less central line. Thus well defined diplococcoid types are produced which appear not unlike small pneumococci. The formation of these diplo forms takes place from twenty-four to ninety-six hours or longer after the pinching off process. Measurements taken with a Filer hair micrometer showed the forms in this particular stage of the development cycle to be from 0.11 to 0.38 micron in diameter. Resubdivision of these bodies then proceeds at a comparatively rapid rate until after a subsequent twenty-four hours or more of incubation the droplet will be found to be well populated with tiny cocci and diplococci, too small to measure and with some appearing as short chains. These are oval, round and often somewhat irregular in outline. Forms taken to be in this stage have been found to be nonacid-fast and are possibly the types described by Much (Beitr. z. klin. d. Tuberk. **8**:357, 1907). An additional day or more in the incubator discloses a well defined tendency toward agglutination. Although there are many free granules, several clumps will be found to have formed, and the particles within these clumps then begin to reduce in size to such an extent that after twenty-four or forty-eight hours of further incubation, tiny groups of dustlike particles are all that remain in the droplet. The individuals in these groups are so small that measurements with the hair micrometer has been found impossible and the forms are distinctly visible only about the periphery of the tufts; many of them are on the outer limit of visibility. It is possible that these individuals may be small enough to be ultramicroscopic, and it is conceivable also that during this stage of its development, the bacillus of tuberculosis may be a filter passer, although experiments to prove this point have not been performed. Much has made reference to the occurrence of tufts of dustlike particles in specimens obtained from tubercular material. These may be similar, if not identical, forms.

These clumps remain as such for varying periods—twenty-four, forty-eight, ninety-six hours or even longer. Eventually, however, the most delicate imaginable rods will be found sprouting from the periphery. At first the rods are so small that one cannot be sure of their identity. They grow with varying rapidity until finally the adult acid-fast rod is produced. Some of these tiny rods break from the parent clump and develop independently at some distance. Most of them, however, remain in the group and complete their development at that locality.

Whether these tiny rods sprout from individual granules, or whether the granules elongate into the delicate filaments is at present unanswerable for want of proper magnification. We have considered at times that these young rods may further divide by direct fission, as branching forms and bent forms have been clearly seen. No such process, however, has been actually observed to take place even when subcultures have been made with a micropipet from the confused germinating mass contained in a single drop.

During the early stages of the development of these rods they appear yellow white under transmitted light. After four or five days, however, they assume a definite greenish hue under the same illumination, a color also held by the mature tubercle bacillus. This difference in light transmission may possibly be due to the formation of the acid-fast coat on the part of the older organisms. Some slight evidence may be gathered from studying stained preparations partially to sub-

stantiate this conception. When stains are made from tufts containing a number of immature rods, a considerable number of definitely nonacid-fast rods are encountered and also those staining a mulberry color, more pink than nonacid-fast and less red than the frankly acid-fast types. The nonacid-fast types vary in size from those too small to measure to forms varying from 0.3 to 1.2 microns. The length of the adult tubercle bacillus measures from 1.2 to 5 microns. Petroff (Tuberculosis, Bacteriology, Pathology and Laboratory Diagnosis, Philadelphia, Lea & Febiger, 1927) claims that nonacid-fast rods are more common when the culture medium is unfavorable or when it does not contain any glycerol. In our microcultures they have been found to be numerous at a certain stage of the development cycle when the culture medium was favorable and contained glycerol, but they may well be present in added numbers on a poor medium in view of lack of sufficient food material to complete the development.

A so-called wild strain of human tubercle bacillus isolated from infected urine has been found to undergo the same development cycle as has been described.

It is entirely possible that the development cycle here induced was due solely to the artificial method of cultivation employed and that the same condition will not be found to take place in the animal body. On the other hand, it must be remembered that a number of investigators have observed elements similar to those described, in material obtained from pathologic sources, and, as has already been pointed out, the absence of demonstrable acid-fast rods does not preclude the possibility of tuberculosis. The importance of animal inoculation is thus particularly emphasized.

DISCUSSION

JOHN C. TORREY: In commenting I wish to say that I have followed Dr. Kahn's observations carefully, and I believe they are sound, and that he has interpreted properly the cycle of the development of the tubercle bacillus from the rod to the granular stage and to the rod again. Not always does the cycle follow just this way, but that is one of the ways in which the tubercle bacillus can undergo development. One of the greatest difficulties encountered was the technical difficulty at the beginning. It took Dr. Kahn several months to devise the cell which would be sufficiently moist so that the exceedingly minute droplets would not evaporate at 37 C. for a period of weeks or months. Dr. Kahn is greatly indebted to Dr. Robert Chambers for his ingenious device for studying single cells.

A number of observers from the time of Koch to the present have noted these granules in the tissues and also in cultures—nonacid-fast granules, and some of them have suggested a possible cycle of development of the tubercle bacillus and the relation of these granules to the tubercle rod as seen in the acid-fast condition. But these were merely surmises. It is impossible to follow the life cycle of the tubercle bacillus, except under viable conditions. Dr. Kahn has been able to do that. I have observed his granules a number of times. One day one would see a small quantity of the granules and the next day rods, exceedingly fine, sprouting from the granules, and finally the development of the tubercle bacillus. Although one may say that the conditions under which the work was done were artificial, we have no right to deny that these different stages may not occur in the human body. In fact, several observers have seen these granules, and nonacid-fast rods in tuberculous lesions entirely like those described by Dr. Kahn, and it is probable that there are tuberculous lesions in which only these granules are present, at least for a time.

ELISE L'ESPERANCE: One can hardly add anything to this excellent paper of Dr. Kahn. I have seen some of the stages he describes in direct smears in tissue from chickens which developed avian tuberculosis. I feel that this work is of far-reaching importance in future investigations, in lymphogranuloma, Hodgkin's disease and in the leukemias, especially, if it is borne out, as I think it will be, that these different stages of the tubercle bacillus occur in the human body.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

HOWARD T. KARSNER, *Secretary*

Twenty-Eighth Annual Meeting, Washington, D. C., May 1, 2 and 3, 1928

J. W. JOBLING, *President*

SPECIAL REPORT

The first session was a joint meeting with the American Association of Immunologists. A. E. Sheplar, M. A. Lyons and Ward J. MacNeal reported observations indicating that the syphilitic reagin is a mixture of various substances. Forest Huddleson described differences in specific agglutination during infection and in immunity. E. W. Schultz reported that while specific neutralizing bodies are present in the serum of rabbits immunized against vaccinia, rabies and herpes, specific fixation or precipitation cannot be demonstrated. Stuart Mudd, Baldwin H. Lucke, Morton McCutcheon and Max Strumia reported that immune rabbit serum alters the surface properties of the corresponding bacteria and thus promotes phagocytosis. Ralph R. Mellon described the relation of the factor of surface tension to spontaneous agglutination and electrophoresis of bacteria. David Perla and J. Marmorston-Gottesman reported that in rats the suprarenals and epinephrine inhibit the formation of hemolysins. John C. Torrey and Morton C. Kahn described the production of an anemia, like pernicious anemia, in animals by the injection of the toxin of *B. welchii*.

At the second session, on the afternoon of May 1, J. Bronfenbrenner and D. Hetler reported that lysis is not an essential part of the phenomenon of bacteriophagy. Adelaide B. Baylis and Ward J. MacNeal described the resorcin flocculation test for active tuberculosis. Max B. Lurie reported on the fate of tubercle bacilli in the various organs of the rabbit in relation to natural and acquired resistance. F. A. Hecker discussed the results of experiments showing that high concentrations of vitamine B stimulate growth of the spleen of the chick in vitro. S. Shields Warren stated that amniotic fluid has the power of preventing adhesions of serous surfaces. Bernhard Steinberg discussed the cellular response to the intraperitoneal injection of colon bacilli and fecal material in animals. Walter M. Simpson presented the report of the autopsy in a rapidly fatal case of tularemia, the lesions of which were like those of an infectious granuloma.

At the third session on Wednesday morning, May 2, Alfred Plaut described a hamartoblastoma of the kidney. N. W. Barker reported on the arteries of the human kidney as studied by the method of celluloid corrosion. Saul A. Ritter and George Baehr reported on the arteriolar changes in primary and secondary contraction of the kidney. Margaret Warwick described a case of nephrosis with glomerulonephritis. Leone McGregor reported on experimental glomerulonephritis produced by the arterial injection of bovine tubercle bacilli. Esmond R. Long, Lucy Finner and Paul J. Patchen announced that glomerulonephritis can be produced in tuberculous swine by arterial perfusion of the kidneys with a fine suspension of the protein of purified tuberculin. Robert A. Moore stated that in mercuric chloride poisoning of white rats changes occur in the mitochondria in the third part of the convoluted tubules after renal insufficiency has been established. Warren C. Hunter found that in the rabbit the renal epithelium may acquire resistance to the toxic action of uranium. James E. Davis reported on the kidneys in a large series of autopsies with a view to correlating the anatomic and the clinical observations. E. T. Bell presented a general discussion of glomerulonephritis, nephrosis and renal arterial sclerosis with a scheme for classifying the nephropathies.

At the fourth session on Thursday morning, May 3, E. C. Rosenow reported on the microscopic changes and bacteriology of experimental poliomyelitis in the monkey. Maude E. Abbott presented the results of a statistical study of the causes of death in persons with coarctation of the aorta (adult type). Eli Moschcowitz discussed arteriosclerosis, with particular reference to intravascular pressure. Ward J. MacNeal described the circulation of the blood through the pulp of the spleen. W. L. Robinson reported that in the cat the venous system of the spleen is not continuous with the arterial system. G. R. Callender reported that in the organization of pneumonic exudate in influenza fibers of collagen and reticulum appear independently. Aldo Castellani discussed metadysentery bacilli, which do not produce gas in any sugar, with particular reference to their clinical relationships. Charles L. Connor described the chemical and microscopic characteristics of the principal animal lipochromes. Lawrence W. Smith and John V. Leech reported four instances of laterally aberrant thyroid tumors and their probable relationship to the so-called fifth pharyngeal pouch. Ernest S. Scott and Harry L. Reinhart described a case of multiple primary tumors of the liver of reticulo-endothelial origin. Percival Bailey discussed tumors and malformation of the blood vessels of the central nervous system. H. Mooser described the changes in the scrotum of guinea-pigs inoculated with Mexican typhus fever; the tunica, which becomes highly infectious, shows swollen endothelial cells containing a great quantity of minute diplococcal forms resembling the cells of the stomach of lice containing *Rickettsia prowazeki.*

Book Reviews

CONTRIBUTIONS TO MEDICAL SCIENCE. THE ALDRED SCOTT WARTHIN ANNIVERSARY VOLUME. DEDICATED TO ALDRED SCOTT WARTHIN, A.M., PH.D., M.D., F.A.C.P., Professor of Pathology and Director of the Pathological Laboratories of the University of Michigan, in honor of his sixtieth birthday and the completion of his thirty-fifth year of teaching in the University, Oct. 21, 1926, by his pupils and early colleagues. Price, $10. Pp. 302, with 275 illustrations. Ann Arbor, Mich.: George Wahr, 1927.

George Dock has aptly stated in his introduction to this magnificent volume, "*Festschriften* are so rarely published in America that the present stately volume would be noteworthy for that reason alone, but this one has the distinction of being dedicated to a veteran teacher of pathology in a great medical school, and so acquires a special importance." The volume contains fifty-four scientific contributions contributed by sixty-four authors. Of this number, Vaughan, Dock, Huber, Novy and Rous are representatives of Warthin's early colleagues. The other writers include one or more from each of the thirty-five successive classes which he has taught. In the latter respect, this volume is unique among medical "Festschriften."

The papers cover such a wide range of medical interests that it is impossible, and would be indeed unwise, to attempt to assign relative values to the papers. Most of them are concerned with topics that are of special interest to pathologists. These will be abstracted individually by the reviewer for later publication in this journal. The broad scope of the undertaking and the catholicity of interests are best indicated by a list of the authors and titles:

Vaughan, Proem; Dock, Aldred Scott Warthin, An Appreciation; Huber, New Method of Fixation and Staining of Central Nervous System for Purpose of Study of Cyto-architecture; Novy and Soule, Some Observations on the Gas Exchange of the Bovine Tubercle Bacillus; Rous, Pathology and the Glare of the Future; Hamilton, Fifteen Years of Industrial Toxicology; McCord, Industrial Benzol (Benzene) Poisoning; Kingery, Fruit Poisoning: An Investigation of Its Etiology, Symptomatology and Pathology with Suggestions as to Treatment; Wiggers, The Cause of Temporary Ventricular Alternation Following a Long Diastolic Pause; Morris, Clinical Notes on Pulsus Alternans; Burnett, Low Voltage in the Electrocardiogram; Herrmann, Situs Inversus Viscerum Totalis, with Electrocardiographic and Roentgenographic Studies for Clinical Differential Diagnosis; Brooks, The Treatment of Cardiovascular Syphilis; Stokes, The Diagnosis and Treatment of Cardiovascular and Visceral Syphilis; Stone, Angina Pectoris and Coronary Occlusion, with Notes on the Vascular Pathology in Coronary Disease; Starry, Syphilis of the Tonsils as Found in Routine Histological Examination; Arneill, Certain Clinical Observations on Goiter; Smith, The Management of Patients with Toxic Goiter; Potter, Persistent Thymus in Exophthalmic Goiter; Fortune, Significance of Kidney Infiltrations in the Gross Pathological Diagnosis of Lymphoblastoma; Hoag, The Administration of Parathyroid Extract to Infants with Idiopathic Tetany and with Rickets; Cowie and Parsons, The Treatment of Hay-Fever and Pollen Asthma by Pollen Extracts and Changes in Local Environment; Mellon, Certain Theoretic and Practical Aspects of the Newer Biology of the Bacteria; Cumming, The Streptococcus, an Ally of Smallpox; Edmunds, Experimental Study of the Treatment of Botulism; DeWitt, The Chemotherapeutic Value of Gold Salts in Experimental Tuberculosis in Guinea-Pigs; Eberbach, Some Pitfalls in the Diagnosis of Renal Tuberculosis; Senear, The Importance of the Tuberculids in Medical Diagnosis; Breidenbach, Artificial Pneumothorax—Its Mechanism and Physiology; Watkins, The Treatment of Lung Abscess; Elmer, A Comparison of Litten's Diaphragmatic

Phenomenon with Fluoroscopic Observations of Diaphragmatic Movements; Smithies, Parasitosis of the Bile Passages and Gallbladder: Thirty-Seven Instances of Protozoiasis and One Instance of Infestation by Necator Americanus; Wallace and Diamond, Further Observations on the Urobilogen Test for Liver Function; McGee, A Study of Bile Secretion in a Patient with Biliary Fistula; Sturgeon, Diverticulum of the Esophagus; Simpson, Aberrant Pancreatic Tissue: An Analysis of 150 Human Cases, with a Report of a New Case; Tenney, Certain Clinical Observations on Gastric Ulcer; Brace, Insulin and Carbohydrate Tolerance; Hyde, Histological Diagnosis by the Surgeon; Haythorn, Experimental Edema as an Aid to Histopathologic Studies; Vaughan, The Reaction of the Omentum to Germ Substance; Wilson, A New Selective Staining Method for the Demonstration of the Glomerular Vascular Bed; MacNeal, The Splenic Lobule; Warren, Gaucher's Disease; Weller, The Phagocytosis of Melanin by the Reticulo-Endothelial Cells in a Case of Melanoblastoma; Barney, Chemical Analysis of Sweat; Badgley, Two Stage Transplantation of the Fibula for the Tibia; Faber, Craniosynostosis (Oxycephaly and Related Disorders); Smith, Total Rhinoplasty; Lillie, Recent Clinical Observations on Involvement of the Blood Stream in Otitic Disease; Canfield and Furstenberg, Clinical Aspects of Laryngeal Cancer; Wieder, A Study of Ill Effects from Lumbar Puncture: Report of a Fatality; Raphael and Dieterle, The Korsakow Syndrome in Association with Epidemic Encephalitis; Garvey, An Extradural Spinal Cord Tumor Syndrome Due to Adenocarcinoma Mucosum of the Parotid Gland with Generalized Metastases; Peet, The Pituitary Adamantinomas; Freund, High Blood Urea, Nonprotein Nitrogen, Creatinine and Uric Acid Values in a Case of Brain Tumor; Weller, Appendix: An Aldred Scott Warthin Bibliography, 1892-1926.

The careful grouping of related topics indicates fine attention to detail on the part of the editor, Willard J. Stone, and the editorial committee, of which Carl V. Weller was chairman. The printing is done on gloss finished book paper, which permits excellent reproduction of the unusually great number of illustrations. This is particularly true of the photomicrographs. The book is handsomely bound with deep blue fabricoid binding, with gilt lettering and top.

Naturally, the book will appeal most strongly to Warthin's colleagues and former students. But the high scientific value of its papers, and the fact that most of the articles will not appear elsewhere, render it an important acquisition for every medical library.

Weller has compiled a complete Warthin bibliography, which forms an appendix to the book. Here approximately 400 contributions are indexed in chronologic sequence.

Warthin's influence on his students is abundantly manifested throughout the work by their scholarly appreciation of fundamental pathology. The volume is a fitting and glorious tribute to one whose fame as a stimulating teacher and scientific investigator will live long. How fortunate that this well merited recognition should come while he is at the height of his productivity!

DIE GEWEBEZÜCHTUNG IN VITRO. By V. BISCEGLIE and A. JUHASZ-SCHÄFFER. (Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere). Volume 14. Price, 28 marks. Pp. 355, with 71 illustrations. Berlin: Julius Springer, 1928.

In this monograph are presented the history, technic and most important results of the work with tissue culture. There are chapters on the technic of tissue culture; general growth phenomena of the explants with a description of the various animal tissues in culture. There are also chapters on the cultivation of plant cells, on growth influencing factors, on the structure of protoplasm and one on the process of mitosis. The last four chapters are devoted to physiologic problems dealing with pathologic processes of inflammation and immunity of tissue extracts, and with filtrable viruses and tumors.

The chapter on technic is somewhat meager and, beyond indicating the general methods used, would not be of great value in helping a new worker in this field. For example, the great saving in time and labor which can be effected by the use of heparin in obtaining plasma is scarcely mentioned.

A fairly good review of the most important results obtained by the use of tissue cultures is presented. As is characteristic of most reviews of such a comprehensive nature, there are some points which are well treated while others — as for instance that dealing with the developmental potencies of the blood and connective tissue cells — are rather poorly done and there are some serious misquotations. Undue prominence has been given to the work with "affronted" tissues, for it has not yet been shown that many of the results obtained by this method could not be explained as individual variations in the cultures.

The bibliography is extensive and relatively free of errors. The illustrations on the whole are well done, although the reproduction of some of the figures of several authors are far inferior to the originals. The photomicrographs are good.

The main purpose of the book is to review the past work and to indicate problems for future experimentation and this has been done well in view of the vast fields covered. The chief criticism of the book can be expressed in saying that too much emphasis and importance is attached to what has been accomplished by the method. Although tissue culture has thrown light on many problems, the great achievements possible by the method are still in the future.

The book gives a better general view of tissue culture than any which has as yet appeared, for it is not so exclusively limited in scope as that of Fischer. It is incomparably inferior, however, to the latter in the consideration of the tissue culture technic. This is the best book on the subject for the investigator who is interested in a general review of tissue culture.

METHODS AND PROBLEMS OF MEDICAL EDUCATION (NINTH SERIES). INSTITUTES OF LEGAL MEDICINES. Pp. 386. New York, The Rockefeller Foundation, 1928.

The volume contains illustrated descriptions of a large number of institutes of legal medicine. The countries represented by established institutes are Austria, Czechoslovakia, Denmark, Egypt, France, Germany, Hungary, Italy, Poland, Portugal, Roumania, Scotland, Sweden and Switzerland. In practically every case the institute described is part of a university and the director, a professor of forensic medicine. The descriptions are by the directors. In some cases the description is accompanied by a more or less exhaustive discussion of the practical workings of the institute and of the medicolegal practices and problems of the country or city in question. This statement applies especially to the medicolegal institutes in Egypt (Sidney Smith), Edinburgh (H. H. Littlejohn), Switzerland (H. Zangger), Denmark (K. Sand) and Sweden (G. Sjövall). The discussion by Zangger (Medizin und Recht, 53 pages) is especially elaborate and philosophic and of wider applicability. Horst Oertel presents a brief but illuminating statement on the academic position of legal medicine in Canadian universities which in its essentials is applicable also to the conditions in the United States. Raimundo de Castro and Antonio Barreras describe the plans proposed for legal medicine in connection with the University of Havana. Timothy Leary discusses the medicolegal system in Massachusetts and submits proposals for a complete pathologic institute in Boston. Leary's paper contains many interesting historical details. Charles Norris writes instructively on the responsibility of the chief medical examiner of New York City in relation to medical progress, education and research. In the papers by Leary and Norris are described the workings and facilities of the two main medicolegal systems in vogue in the United States that are different from the coroner's system. There is no description or report presented of specific conditions and operation under the coroner's system. This volume, which is distributed free, should have a wide distribution among physicians, lawyers and public officials who are interested in promoting much needed improvement and progress in the field of legal medicine in the United States.

Books Received

THE NEWER KNOWLEDGE OF BACTERIOLOGY AND IMMUNOLOGY. By Eighty-Two contributors. Edited by Edwin O. Jordan and I. S. Falk, the University of Chicago. Price, $10. Pp. 1133. Chicago: The University of Chicago Press, 1928.

DER KREBS DES MENSCHEN. EINE MORPHOGENETISCHE Untersuchung. Von Dr. Eugen Bostroem, Em. Professor in Giessen. Price, 12 marks. Pp. 176, with 17 illustrations. Leipzig: Georg Thieme, 1928.

KLINISCHE LABORATORIUMSTECHNIK. Herausgegeben von Professor Dr. Theodor Brugsch, Direktor der Medizinischen Klinik Halle a.S. und Professor Dr. Alfred Schittenhelm, Direktor der Medizinischen Klinik Kiel. III. Band. Unter Mitarbeit von K. Beckmann, P. Bohnen, M. Bürger, F. Chrometzka, H. Erbsen, Rh. Erdmann, W. Frey, M. Pappis, E. Kylin, B. Niekau, V. van der Reis, K. Retzlaff, F. Schellong, A. Schittenhelm, C. Sonne, H. Ucko, E. Weih, H. Zondek. Second edition. Price, 32 marks. Pp. 1449-2100, with 144 illustrations, and 1 colored and 1 black and white plate. Berlin: Urban & Schwarzenberg, 1928.

EXPERIMENTELLE NEUROLOGIE (PHYSIOLOGIE UND PATHOLOGIE DES NERVENSYSTEMS). Von E. A. Spiegel. Privatdozent an der Universität Wien, Assistent am Neurologischen Institut. I. Teil. Price, 24 marks. Pp. 281, with 69 colored illustrations. Berlin: Von S. Karger, 1928.

METHODS AND PROBLEMS OF MEDICAL EDUCATION (NINTH SERIES). Institutes of Legal Medicines. Pp. 386. New York: Division of Medical Education, The Rockefeller Foundation, 1928.

ELEMENTARY BACTERIOLOGY. By Joseph E. Greaves, M.S., Ph.D., Professor of Bacteriology, Utah Agricultural College, and Ethelyn O. Greaves, M.S. Price, $3.50. Pp. 506, with 129 illustrations. Philadelphia: W. B. Saunders Company, 1928.

PHYSIOLOGY AND BIOCHEMISTRY OF BACTERIA. Volume 1. Growth Phases, Composition and Biophysical Chemistry of Bacteria and Their Environment; Energetics. By R. E. Buchanan, Iowa State College, and Ellis I. Fulmer, Iowa State College. Price, $7.50. Pp. 516. Baltimore: Williams & Wilkins Company, 1928.

KONSTITUTIONSPATHOLOGIE IN DER ORTHOPÄDIE ERBBIOLOGIE DES PERIPHEREN BEWEGUNGSAPPARATES. Von Dr. Berta Aschner und Dr. Guido Engelmann, Privatdozen. Price, 28 marks. Pp. 312, with 80 illustrations. Berlin: Julius Springer, 1928.

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